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(72) Inventeurs/Inventors:
LAW, CHE-LEUNG, US;
MCEARCHERN, JULIE, US;
WAHL, ALAN F., US

(73) Propriétaire/Owner:
SEATTLE GENETICS, INC., US

(74) Agent: SMART & BIGGAR

(54) Titre : ANTICORPS ANTI-CD70 ET SON UTILISATION POUR LE TRAITEMENT ET LA PREVENTION DU CANCER ET DES TROUBLES IMMUNITAIRES

(54) Title: ANTI-CD70 ANTIBODY AND ITS USE FOR THE TREATMENT AND PREVENTION OF CANCER AND IMMUNE DISORDERS

(57) Abrégé/Abstract:
Disclosed are CD70 binding agents, such as anti-CD70 antibodies and derivatives, that are cytotoxic, cytostatic or immunosuppressive without conjugation to a therapeutic agent, as well as pharmaceutical compositions and kits comprising the antibody or derivative. Also disclosed are methods for the treatment and prevention of CD70-expressing cancers and immunological disorders comprising administering to a subject the CD70-binding agent. In selected ebodiments, CD70 binding agents have an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject, for use in treatment of a CD70-expressing cancer.

ANTI-CD70 ANTIBODY AND ITS USE FOR THE TREATMENT AND PREVENTION OF CANCER AND IMMUNE DISORDERS

ABSTRACT OF THE DISCLOSURE

Disclosed are CD70 binding agents, such as anti-CD70 antibodies and derivatives, that are cytotoxic, cytostatic or immunosuppressive without conjugation to a therapeutic agent, as well as pharmaceutical compositions and kits comprising the antibody or derivative. Also disclosed are methods for the treatment and prevention of CD70-expressing cancers and immunological disorders comprising administering to a subject the CD70-binding agent. In selected ebodiments, CD70 binding agents have an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject, for use in treatment of a CD70-expressing cancer.

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ANTI-CD70 ANTIBODY AND ITS USE FOR THE TREATMENT AND PREVENTION OF CANCER AND IMMUNE DISORDERS

CROSS REFERENCES TO RELATED APPLICATIONS

[0001] <deleted>

BACKGROUND OF THE INVENTION

[0002] CD70 is a member of the tumor necrosis factor (TNF) family of cell membrane-bound and secreted molecules that are expressed by a variety of normal and malignant cell types. The primary amino acid (AA) sequence of CD70 predicts a transmembrane type II protein with its carboxyl terminus exposed to the outside of cells and its amino terminus found in the cytosolic side of the plasma membrane (Bowman *et al.*, 1994, *J. Immunol.* 152:1756-61; Goodwin *et al.*, 1993, *Cell* 73:447-56). Human CD70 is composed of a 20 AA cytoplasmic domain, an 18 AA transmembrane domain, and a 155 AA extracytoplasmic domain with two potential N-linked glycosylation sites (Bowman *et al.*, *supra*; Goodwin *et al.*, *supra*). Specific immunoprecipitation of radioisotope-labeled CD70-expressing cells by anti-CD70 antibodies yields polypeptides of 29 and 50 kDa (Goodwin *et al.*, *supra*; Hintzen *et al.*, 1994, *J. Immunol.* 152:1762-73). Based on its homology to TNF-alpha and TNF-beta, especially in structural strands C, D, H and I, a trimeric structure is predicted for CD70 (Petsch *et al.*, 1995, *Mol. Immunol.* 32:761-72).

[0003] Original immunohistological studies revealed that CD70 is expressed on germinal center B cells and rare T cells in tonsils, skin, and gut (Hintzen *et al.*, 1994, *Int. Immunol.* 6:477-80). Subsequently, CD70 was reported to be expressed on the cell surface of recently antigen-activated T and B lymphocytes, and its expression wanes after the removal of antigenic stimulation (Lens *et al.*, 1996, *Eur. J. Immunol.* 26:2964-71; Lens *et al.*, 1997, *Immunology* 90:38-45). Within the lymphoid system, activated natural killer cells (Orengo *et al.*, 1997, *Clin. Exp. Immunol.* 107:608-13) and mouse mature peripheral dendritic cells (Akiba *et al.*, 2000, *J. Exp. Med.* 191:375-80) also express CD70. In non-lymphoid lineages, CD70 has been detected on thymic medullar epithelial cells (Hintzen *et al.*, 1994, *supra*; Hishima *et al.*, 2000, *Am. J. Surg. Pathol.* 24:742-46).

[0004] In addition to expression on normal cells, CD70 expression has been reported in different types of cancers including lymphomas, carcinomas, and tumors of neural origin. In malignant B cells, 71% of diffuse large B-cell lymphomas, 33% of follicle center lymphomas, 25% of mantle lymphomas, and 50% of B-CLL have been reported to express CD70 (Lens *et al.*, 1999, *Br. J. Haematol.* 106:491-503). CD70 is frequently expressed together with other lymphoid activation markers on the malignant Hodgkin and Reed-Sternberg cells of Hodgkin's disease (Gruss and Kadin, 1996, *Bailieres Clin. Haematol.* 9:417-46). One report demonstrates CD70 expression on 88% (7 of 8 cases) of thymic carcinomas and 20% (1 of 5 cases) of atypical thymomas (Hishima *et al.*, 2000, *supra*). The second type of carcinoma on which CD70 has been detected is nasopharyngeal carcinoma. One study reports the presence of CD70 on 80% (16 of 20 cases) of snap-frozen tumor biopsies obtained from undifferentiated nasopharyngeal carcinomas (Agathanggelou *et al.*, 1995, *Am J Path* 147:1152-60). CD70 has also been detected on brain tumor cells, especially glioma cell lines, solid human gliomas, and meningiomas (Held-Feindt and Mentlein, 2002, *Int. J. Cancer* 98:352-56; Wischlusen *et al.*, 2002, *Can. Res.* 62:2592-99).

[0005] The receptor for CD70 is CD27, a glycosylated type I transmembrane protein of about 55kDa (Goodwin *et al.*, 1993, *Cell* 73:447-56; Hintzen *et al.*, 1994, *supra*). CD70 is sometimes referred to as CD27L. CD27, which exists as a homodimer on the cell surface (Gravestein *et al.*, 1993, *Eur. J. Immunol.* 23:943-50), is a member of the TNF receptor superfamily as defined by cysteine-rich repeats of about 40 amino acids in the extracellular domain (Smith *et al.*, 1990, *Science* 248:1019-23; Locksley *et al.*, 2001, *Cell* 104:487-501). CD27 is expressed by thymocytes, NK, T, and B cells (Hintzen *et al.*, 1994, *Immunol Today* 15:307-11; Lens *et al.*, 1998, *Semin. Immunol.* 10:491-99). On resting T cells, CD27 is constitutively expressed, yet antigenic triggering further upregulates CD27 expression (de Jong *et al.*, 1991, *J. Immunol.* 146:2488-94; Hintzen *et al.*, 1993, *J. Immunol.* 151:2426-35). Further, triggering of T cells via their T cell antigen receptor complex alone or in combination with the accessory molecule CD28 releases soluble CD27 from activated T cells (Hintzen *et al.*, 1991, *J. Immunol.* 147:29-35). Naïve B cells do not express CD27, but its expression is induced and, in contrast to CD70, sustained after antigenic triggering of B cells (Jacquot *et al.*, 1997, *J. Immunol.* 159:2652-57; Kobata *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* 92:11249-53).

[0006] In marked contrast to the restricted expression of CD27 and CD70 in normal B lineage cells, both CD27 and CD70 are frequently co-expressed in many B cell non-Hodgkin's lymphomas and leukemias. This could potentially lead to functional CD27-CD70 interactions on these cells in the form of an autocrine loop, resulting in CD27 signaling and in CD70-induced proliferation, thereby providing a growth advantage to malignant cells (Lens *et al.*, 1999, *supra*).

[0007] The role of CD70-CD27 co-stimulation in cell-mediated autoimmune diseases has been investigated in a model of experimental autoimmune encephalomyelitis (EAE) (Nakajima *et al.*, 2000, *J. Neuroimmunol.* 109:188-96). *In vivo* administration of the anti-mouse CD70 mAb (clone FR-70) suppressed the onset of EAE by inhibiting antigen-induced TNF-alpha production without affecting B and T cell number, T cell priming, Ig production or T_H1/T_H2 cell balance. However, such treatment had little efficacy in established disease.

[0008] Graft versus host disease (GVHD) is aT_H1-mediated immune response that is a major and often lethal consequence of allogeneic bone marrow transplantation (BMT) therapy that occurs when histocompatibility antigen differences between the BM donor and the recipient of the transplant are present (den Haan *et al.*, 1995, *Science* 268:1476). GVHD is an immune reaction against host tissues mounted by mature T cells present in the transplanted donor marrow (Giralt and Champlin, 1994, *Blood* 84:3603). It is noteworthy that CD70 has been detected *in vivo* on CD4+ cells in conditions characterized by allogeneic reaction, as in cases of maternal T cell engraftment in severe combined immune deficiency patients (Brugnoni *et al.*, 1997 *Immunol. Lett.* 55:99-104). Prophylaxis of GVHD is achieved by pan-T cell immunosuppressive agents such as cyclosporine, corticosteroids, or methotrexate. Howver, these agents are not specific and cause significant adverse side effects.

[0009] As indicated *supra*, CD70 is not expressed on normal non-hematopoietic cells. CD70 expression is mostly restricted to recently antigen-activated T and B cells under physiological conditions, and its expression is down-regulated when antigenic stimulation ceases. Evidence from animal models suggests that CD70 may contribute to immunological disorders such as, *e.g.*, rheumatoid arthritis (Brugnoni *et al.*, 1997, *Immunol. Lett.* 55:99-104), psoriatic arthritis (Brugnoni *et al.*, 1997, *Immunol. Lett.* 55:99-104), and lupus (Oelke *et al.*, 2004, *Arthritis Rheum.* 50:1850-60). In addition to its potential role in inflammatory responses, CD70 is also expressed on a variety of transformed cells including lymphoma B

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cells, Hodgkin and Reed-Sternberg cells, malignant cells of neural origin, and a number of carcinomas.

[0010] Accordingly, there is a need for anti-CD70 antibodies and other CD70 binding agents that can exert a clinically useful cytotoxic, cytostatic, or immunosuppressive effect on CD70-expressing cells, particularly without exerting undesirable effects on non-CD70-expressing cells. Such binding agent would be useful against cancers that express CD70 or immune disorders that are mediated by CD70-expressing cells.

BRIEF SUMMARY OF THE INVENTION

[0011] The present invention provides CD70 antibodies and other CD70 binding agents and methods relating to the use of such binding agents for the prophylaxis or treatment of CD70-expressing cancers and immunological disorders where CD70-expressing cells are present. The antibody or other binding agent binds to CD70 and exhibits a cytotoxic, cytostatic, and/or immunosuppressive effect on CD70-expressing cells in the absence of conjugation to a therapeutic agent.

[0012] In one aspect, a method of treating a CD70-expressing cancer in a subject is provided. The method generally includes administering to the subject an effective amount of a binding agent having an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject, wherein the binding agent exerts a cytostatic or cytotoxic effect in the absence of conjugation to a therapeutic agent. The CD70-binding agent can be, for example, an antibody, such as a chimeric, humanized, or fully human antibody. The antibody can include, for example, an effector domain of a human IgM or IgG antibody. The IgG antibody can be, for example, a human IgG1or IgG3 subtype. In some embodiments, the antibody includes a human constant region.

[0013] In some embodiments, the antibody competes for binding to CD70 with monoclonal antibody 1F6 or 2F2. In other embodiments, the antibody is a humanized 1F6 or 2F2 or a chimeric 1F6 or 2F2 antibody. The antibody can be, for example, monovalent, divalent or multivalent.

[0014] The CD70-expressing cancer can be, for example, a kidney tumor, a B cell lymphoma, a colon carcinoma, Hodgkin's Disease, multiple myeloma, non-Hodgkin's

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lymphoma, chronic lymphocitic leukemia, acute lymphatic leukemia, a nasopharyngeal carcinoma, brain tumor or a thymic carcinoma. The kidney tumor can be, for example, a renal cell carcinoma. The brain tumor can be, for example, a glioma, a glioblastoma, or a meningioma. The subject can be, for example, a mammal, such as a human being.

[0015] In another aspect, a method for treating an immunological disorder is provided. The method includes administering to a subject an effective amount of a binding agent having an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject, wherein the binding agent exerts a cytostatic, cytotoxic, or immunosuppressive effect in the absence of conjugation to a therapeutic agent. The CD70 binding agent can be, for example, an antibody, such as a chimeric, humanized, or fully human antibody. The antibody can include,for example, an effector domain of a human IgM or IgG antibody. The IgG antibody can be, for example, a human IgG1or IgG3 subtype. In some embodiments, the antibody includes a human constant region.

[0016] In some embodiments, the antibody competes for binding to CD70 with monoclonal antibody 1F6 or 2F2. In other embodiments, the antibody is a humanized 1F6 or 2F2 or a chimeric 1F6 or 2F2 antibody. The antibody can be, for example, monovalent, divalent or multivalent.

[0017] The immunological disorder can be, for example, a T cell-mediated immunological disorder. In some embodiments, the T cell mediated immunogical disorder comprises activated T cells expressing CD70. In some embodiments, resting T cells are not substantially depleted by administration of the binding agent. The T cell-mediated immunological disorder also can be, for example, rheumatoid arthritis, systemic lupus E (SLE), Type I diabetes, asthma, atopic dermitus, allergic rhinitis, thrombocytopenic purpura, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease. In other embodiments, the immunological disorder is an activated B-lymphocyte disorder. The subject can be, for example, a mammal, such as a human being.

[0018] In another aspect, an antibody includes an antigen-binding region that binds to CD70 is provided. The antibody includes at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject, and exerts a cytostatic or cytotoxic effect

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on a CD70 expressing cancer, which cytostatic or cytotoxic effect is achieved in the absence of conjugation to a cytostatic or cytotoxic agent, and wherein the antibody is not monoclonal antibody 1F6 or 2F2. The antibody can compete for binding to CD70 with monoclonal antibody 1F6 and 2F2.

[0019] In another aspect, the antibody includes an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADC, ADCC, ADCP or CDC response in a subject, and exerts an immunosuppressive effect on a CD70 expressing immunological disorder, which immunosuppressive effect is achieved in the absence of conjugation to a cytostatic or cytotoxic agent, and wherein the antibody is not monoclonal antibody 1F6 or 2F2. The antibody can compete for binding to CD70 with monoclonal antibody 1F6 and 2F2.

[0020] In a related aspect, also provided is a pharmaceutical composition for the treatment of a CD70-expressing cancer or an immunological disorder. The composition includes a CD70-binding antibody and at least one pharmaceutically compatible ingredient. Further provided is a pharmaceutical kit including a container including a CD70-binding antibody, wherein the antibody is lyophilized, and a second container comprising a pharmaceutically acceptable diluent.

[0020a] Various embodiments of the present invention relate to use of a humanized or chimeric antibody for the treatment of a CD70-expressing cancer in a subject, wherein the antibody comprises an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first complementarity determining region (CDR) having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human

IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

[0020b] Various embodiments of the present invention relate to use of a humanized or chimeric antibody in the manufacture of a medicament for the treatment of a CD70-expressing cancer in a subject, wherein the antibody comprises an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP, or CDC response in the subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

[0020c] Various embodiments of the present invention relate to use of a humanized or chimeric antibody for the treatment of an immunological disorder characterized by expression of CD70 on immune cells in a subject, wherein the antibody comprises: an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

[0020d] Various embodiments of the present invention relate to use of a humanized or chimeric antibody in the manufacture of a medicament for the treatment of an immunological

disorder characterized by expression of CD70 on immune cells in a subject, wherein the antibody comprises an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP, or CDC response in the subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

[0020e] Various embodiments of the present invention provide a humanized or chimeric antibody comprising an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect on a CD70 expressing cancer, which cytotoxic effect is achieved in the absence of conjugation to a cytotoxic agent.

[0020f] Various embodiments of the present invention provide a humanized or chimeric antibody comprising an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject, wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10,

and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect on a CD70 expressing immune cell, which cytotoxic effect is achieved in the absence of conjugation to a cytotoxic agent.

[0020g] Various embodiments of the present invention provide a pharmaceutical composition for the treatment of a CD70-expressing cancer or an immunological disorder characterized by CD70-expressing immune cells, the composition containing an antibody as defined above and at least one pharmaceutically compatible ingredient.

[0020h] Various embodiments of the present invention provide a pharmaceutical kit comprising: a container comprising an antibody as defined above, wherein the antibody is lyophilized, and a second container comprising a pharmaceutically acceptable diluent.

[0021] The present invention may be more fully understood by reference to the following detailed description of the invention, non-limiting examples of specific embodiments of the invention and the appended figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1. The 1F6 V_L and V_H cDNA and amino acid sequences. The coding and amino acid sequences for the light (VL, upper 2 panels; SEQ ID NOs:11 and 12) and heavy chain (VH, lower 2 panels; SEQ ID NOs:1 and 2) variable regions of 1F6 were determined. The complementarity determining regions (CDRs) for the V_L and V_H were identified according to criteria described in Kabat *et al.* (1991, *Sequences of Proteins of Immunogical Interest*, Washington, DC, US Department of Health and Public Services; Chothia and Lesk, 1987, *J. Mol. Biol.* 196: 901-17). Amino acid residues corresponding to the CDRs are underlined. The signal peptide for the V_L and V_H are identified to be amino residues –20 to -1 and –19 to -1, respectively.

[0023] Figure 2. The 2F2 V_L and V_H cDNA and amino acid sequences. The coding and amino acid sequences for the light (VL, upper 2 panels; SEQ ID NOs:31 and 32) and heavy chain (VH, lower 2 panels; SEQ ID NOs:21 and 22) variable regions of 2F2 were determined. The complementarity determining regions (CDRs) for the V_L and V_H were identified according to criteria described in Kabat *et al.*, *supra*; Chothia and Lesk, *supra*. Amino acid residues corresponding to the CDRs are underlined. The signal peptides for the V_L and V_H were identified to be amino residues -20 to -1 and -19 to -1, respectively.

[0024] Figure 3. Amino acid sequence comparisons between the 1F6 and 2F2 CDRs (SEQ ID NOs:16, 36, 18, 38, 20, 40, 6, 26, 8, 28, 10 and 30, respectively). The amino acid sequences of 1F6 and 2F2 CDRs are aligned. Underlined residues represent conservative substitutions and boxed and italic residues represent divergent substitutions.

[0025] Figure 4. Chimeric 1F6 Expression Vector pDEF14-1F6. The structure of an expression vector for expression of antibodies is shown.

[0026] Figure 5. Chimeric 1F6 anti-CD70 antibody mediates antibody-dependent cellular cytotoxicity (ADCC). $Na_2{}^{51}CrO_4$-labeled target cells (WIL2-S B lymphoblastoid cells, Caki-1 renal cell carcinoma cells, and 786-0 renal cell carcinoma cells) were coated with chimeric 1F6 (c1F6), murine 1F6 (m1F6), or human IgG (hIgG) and mixed with peripheral blood mononuclear cells (PBMC) at an effector to target ratio of 30 $CD16^+$ cells to 1 target cell. After 4 hours, the supernatants from lysed cells were measured on a scintillation counter. The percent specific lysis was calculated as {(test sample cpm – spontaneous cpm) ÷ (total cpm – spontaneous cpm)} X 100. Points represent the mean ± standard deviation of triplicate samples.

[0027] Figure 6. Chimeric 1F6-coated target cells recognized by PBMC from multiple donors. $Na_2{}^{51}CrO_4$-labeled Caki-1 renal cell carcinoma cells were coated with varying concentrations of chimeric 1F6 or non-binding control human IgG (hIgG) and mixed with PBMC from two normal donors (2051661 and ND016) at an effector to target cell ratio of 17 $CD16^+$ cells to 1 target cell. Specific lysis was assessed by measuring chromium-51 activity in culture supernatants four hours later as described in Figure 5.

[0028] Figure 7. Chimeric 1F6 mediates ADCC against lymphoid cell lines. $CD70^+$ B lymphoblastoid cells (WIL2-S) and cutaneous T cell lymphoma cells (HH) were labeled with $Na_2{}^{51}CrO_4$ then mixed with chimeric 1F6 or human Ig (hIgG) at various concentrations as

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indicated. PBMC-containing $CD16^+$ cells were added to the target cells at a ratio of 18:1 ($CD16^+$ cells:target) and percent lysis determined after a four-hour incubation as described in Figure 5.

[0029] Figure 8. Chimeric 1F6 mediates ADCC against $CD70^+$ multiple myeloma cell lines. (A) Expression of CD70 by multiple myeloma cell lines. L-363, JJN-3, LP-1 and U-266 cells were stained with a murine anti-CD70 antibody (open histograms) or a non-binding murine IgG control antibody (solid histograms). Antibody binding was detected with FITC-conjugated anti-mouse IgG and the cells analyzed by flow cytometry. (B) ADCC activity of c1F6. $CD38^+/CD138^+/CD70^+$ multiple myeloma cell lines were labeled with $Na_2{}^{51}CrO_4$ and then mixed with chimeric 1F6 (solid squares) or human Ig (solid triangle) at various concentrations as indicated. $CD16^+$ cells enriched from PBMC were added to the target cells at a ratio of 15:1 ($CD16^+$ cells:target) and the percent lysis determined after a four-hour incubation as described in Figure 5. ADCC activity was blocked by pre-incubating the $CD16^+$ effector cells with antibody to FcγRIII (CD16, open squares). Numbers within each graph indicate the number of CD70 molecules expressed by each cell lines estimated using the QIFIKIT® (DakoCytomation, Carpinteria, CA)

[0030] Figure 9. Chimeric 1F6 mediates ADCC against Hodgkin's disease (HD) cell lines. $CD70^+$ HD cell lines Hs445 and L428 were labeled with $Na_2{}^{51}CrO_4$ then mixed with chimeric 1F6 or human Ig (hIgG) at concentrations indicated. PBMC-containing $CD16^+$ cells were added to the target cells at a ratio of 18:1 ($CD16^+$ cells:target) and percent lysis determined after a four-hour incubation as described in Figure 5. Numbers within each graph indicate the number of CD70 molecules expressed by each cell lines estimated using the QIFIKIT® (DakoCytomation, Carpinteria, CA)

[0031] Figure 10. CD70 induced during antigen-specific T cell expansion. PBMCs from a normal HLA-A0201 donor were stimulated with the M1 peptide derived from the influenza virus matrix protein. (A) and B show a representative example of specific CD70 induction on the expanding $CD8^+/V\beta17^+$ after stimulation with the M1 peptide for five days. Binding of the control IgG (open curves) and anti-CD70 mAb (closed curves) on the $CD8^+/V\beta17^-$ or $CD8^+/V\beta17^+$ cells are shown.

[0032] Figure 11. Dose response comparison of c1F6 on depletion of antigen-specific $CD8^+/V\beta17^+$ cells. PBMCs from a normal HLA-A0201 donor were stimulated with the M1

peptide as described in Figure 10. Peptide-stimulated cultures were untreated or initiated with concurrent addition of irrelevant control mAb, murine anti-CD70 antibody (m1F6) or graded doses of chimeric anti-CD70 antibody (c1F6), as indicated. The percent $CD8^+/V\beta17^+$ cells after 9 days was determined by flow cytometry.

[0033] Figure 12. Chimeric 1F6 mediates complement-dependent cytotoxicity in $CD70^+$ B cells. $CD70^+$ lymphoblastic NHL line (MHH-PREB-1), EBV- Burkitt's lymphoma line (MC116), lymphoblastoid B cell line (WIL2-S), and multiple myeloma cell line (LP-1) were incubated with graded doses of the indicated antibodies in the presence of 10% normal human serum. For MHH-PREB-1, MC116 and WIL2-S antibodies were used at 50, 5, 0.5, and 0.05 μg/mL, while for LP-1 antibodies were used at 50, 10, 2, and 0.4 μg/mL. Human IgG (hIgG) was used as a non-binding negative control antibody. Cell lysis was assayed by cell permeability to the DNA dye, propidium iodide detected by flow cytometry. Background cell lysis in medium only was subtracted to give specific cell lysis.

[0034] Figure 13. Chimeric 1F6 mediates complement-dependent cytotoxicity in $CD70^+$ T cells. c1F6-mediated CDC of the CD70+ cutaneous T cell lymphoma line HH and a $CD70^+$ activated normal T cell line (C9D) was evaluated as described in Figure 12.

[0035] Figure 14. Chimeric 1F6 mediates antibody-dependent cellular phagocytosis (ADCP) against $CD70^+$ cells. $CD70^+$ lymphoblastoid cells (WIL2-S) were labeled with a green fluorescent cell membrane dye (PKH67), treated with graded doses of c1F6 then mixed with monocyte-derived macrophages. After two hours, the mixture was incubated with a PE-conjugated anti-CD11b antibody to label the macrophage surface. Uptake of antibody-coated target cells by macrophages was determined by flow cytometric analysis of green and red double fluorescent cells. For fluorescent microscopy, $CD11b^+$ cells were additionally stained with Alexa Fluor™568 goat anti-mouse IgG to enhance the red signal. (A) WIL2-S cells were treated with control antibody (hIgG1) or c1F6 and mixed with macrophages. The percent phagocytic cells (of total macrophages) that ingested antibody-coated target cells are indicated in the upper right quadrant. (B) WIL2-S cells were treated with graded doses of c1F6 (triangles) or nonbinding control Ig (hIgG1, circle) and the percent of phagocytic cells that ingested target cells was determined by flow cytometry.

[0036] Figure 15. Chimeric 1F6 mediates ADCP against multiple $CD70^+$ cell targets. The indicated $CD70^+$ lymphoma, multiple myeloma, and renal cell carcinoma cell lines were used

as targets for chimeric 1F6-mediated ADCP assays as described in Figure 15. Percentage specific ADCP activity at saturating concentrations of chimeric 1F6 is tabulated.

[0037] Figure 16. In vivo antitumor activity of c1F6 in CD70+ xenograft lymphoma models. SCID mice (n=10/group) were inoculated intravenously with 1 x 10^6 Ramos cells or IM-9 cells one day prior to drug treatment. A single dose of chimeric 1F6 was administered at 1 or 4 mg/kg and a single dose of the non-binding control antibody (IgG) was administered at 4 mg/kg. Survival was monitor and difference between treatment groups was compared using the log-rank test as indicated by the *P* values.

DETAILED DESCRIPTION OF THE INVENTION

[0038] The present invention provides CD70 binding agent and methods for using such binding agents for the the prophylaxis or treatment of CD70-expressing cancers and immunological disorders. The CD70 binding agent includes domain that binds to CD70 (*e.g.*, the extracellular domain) and an effector domain. The present inventors have discovered that a CD70 binding agent containing an effector domain can induces a cytotoxic, cytostatic, or immunosuppressive effect on CD70-expressing cells in the absence of conjugation to a therapeutic agent. The cytotoxic, cytostatic, or immunosuppressive effect can be induced, for example, by recruiting and activating cytotoxic white blood cells, *e.g.*, natural killer (NK) cells, phagocytotic cells (*e.g.*, macrophages) and/or serum complement components.

[0039] In one aspect, the methods and compositions relate to antibodies and antibody derivatives that bind to CD70. In an exemplary embodiment, the antibodies or derivatives thereof compete with monoclonal antibody 1F6 or 2F2 for binding to CD70. A cytotoxic, cytostatic, and/or immunosuppressive effect is mediated by the CD70 antibody or derivative and effector cells or complement components that interact with an effector domain (*e.g.*, an Fc region) of the antibody. The cytotoxic, cytostatic, and/or immunosuppressive effect depletes or inhibits the proliferation of CD70-expressing cells. CD70 antibodies can be monoclonal, chimeric, humanized, and human antibodies. In some embodiments, the antibody constant regions are of the IgG subtype. In some embodiments, the antibody is not a mouse monoclonal antibody.

[0040] In another aspect, the methods and compositions relate to other CD70-binding agents that bind to CD70. The CD70-binding agent binds to an extracellular domain of

CD70. A cytotoxic, cytostatic, and/or immunosuppressive effect is mediated by the CD70-binding agent and effector cells or complement components that interact with an effector domain (*e.g.*, an Fc region). The cytotoxic, cytostatic, and/or immunosuppressive effect depletes or inhibits the proliferation of CD70-expressing cells. CD70-binding agents can be, for example, CD27 and derivatives thereof.

I. Definitions and Abbreviations

[0041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art pertinent to the methods and compositions described. As used herein, the following terms and phrases have the meanings ascribed to them unless specified otherwise.

[0042] The terms "inhibit" or "inhibition of" as used herein means to reduce by a measurable amount, or to prevent entirely.

[0043] The term "CD 70 binding agent" as used herein means an anti-CD70 antibody, a derivative of an anti-CD70 antibody, or other agent that binds to CD70, such as an extracellular domain or a portion thereof.

[0044] A "therapeutic agent" is an agent that exerts a cytotoxic, cytostatic, or immunosuppressive effect on cancer cells or activated immune cells.

[0045] A "cytotoxic effect" refers to the depletion, elimination and/or the killing of a target cell. A "cytotoxic agent" refers to an agent that has a cytotoxic effect on a cell.

[0046] A "cytostatic effect" refers to the inhibition of cell proliferation. A "cytostatic agent" refers to an agent that has a cytostatic effect on a cell (or a specific subset of cells), thereby inhibiting the growth and/or expansion of the cell (or specific subset of cells).

[0047] The term "deplete," in the context of the effect of a CD70-binding agent on CD70-expressing cells, refers to a reduction or elimination of the CD70-expressing cells.

[0048] The term "immunosuppressive agent" as used herein refers to an agent that inhibits the development or maintenance of an immunologic response. Such inhibition can be effected by, for example, elimination of immune cells (*e.g.*, T or B lymphocytes); induction or generation of immune cells that can modulate (*e.g.*, down-regulate) the functional capacity

of other cells; induction of an unresponsive state in immune cells (*e.g.*, anergy); or increasing, decreasing or changing the activity or function of immune cells, including, for example, altering the pattern of proteins expressed by these cells (*e.g.*, altered production and/or secretion of certain classes of molecules such as cytokines, chemokines, growth factors, transcription factors, kinases, costimulatory molecules or other cell surface receptors, and the like). In typical embodiments, an immunosuppressive agent has a cytotoxic or cytostatic effect on an immune cell that promotes an immune response.

[0049] "Immune cell" as used herein refers to a cell of hematopoietic lineage involved in regulating an immune response. In typical embodiments, an immune cell is a T lymphocyte, a B lymphocyte, an NK cell, a monocyte/macrophage, or a dendritic cell.

[0050] The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of a product; thus, "peptides" and "proteins" are included within the definition of a polypeptide. Also included within the definition of polypeptides are "antibodies" as defined herein. A "polypeptide region" refers to a segment of a polypeptide, which segment may contain, for example, one or more domains or motifs (*e.g.*, a polypeptide region of an antibody can contain, for example, one or more complementarity determining regions (CDRs)). The term "fragment" refers to a portion of a polypeptide typically having at least 20 contiguous or at least 50 contiguous amino acids of the polypeptide. A "derivative" is a polypeptide or fragment thereof having one or more non-conservative or conservative amino acid substitutions relative to a second polypeptide; or a polypeptide or fragment thereof that is modified by covalent attachment of a second molecule such as, *e.g.*, by attachment of a heterologous polypeptide, or by glycosylation, acetylation, phosphorylation, and the like. Further included within the definition of "derivative" are, for example, a polypeptides containing one or more analogs of an amino acid (*e.g.*, unnatural amino acids and the like), polypeptides with unsubstituted linkages, as well as other modifications known in the art, both naturally and non-naturally occurring.

[0051] The term "antibody" as used herein refers to (a) immunoglobulin polypeptides and immunologically active portions of immunoglobulin polypeptides (*i.e.*, polypeptides of the immunoglobulin family, or fragments thereof, that contain an antigen binding site that immunospecifically binds to a specific antigen (*e.g.*, CD70)), or (b) conservatively substituted derivatives of such immunoglobulin polypeptides or fragments that

immunospecifically bind to the antigen (*e.g.*, CD70). Antibodies are generally described in, for example, Harlow and Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1988).

[0052] In the context of immunoglobulin polypeptides or fragments thereof as defined above, "conservative substitution" means one or more amino acid substiutions that do not substantially reduce specific binding (*e.g.*, as measured by the K_D) of the immunoglobulin polypeptide or fragment thereof to an antigen (*i.e.*, substitutions that increase binding, that do not significantly alter binding, or that reduce binding by no more than about 40%, typically no more than about 30%, more typically no more than about 20%, even more typically no more than about 10%, or most typically no more than about 5%, as determined by standard binding assays such as, *e.g.*, ELISA).

[0053] An "antibody derivative" as used herein refers to an antibody, as defined above, that is modified by covalent attachment of a heterologous molecule such as, *e.g.*, by attachment of a heterologous polypeptide, or by glycosylation, acetylation or phosphorylation not normally associated with the antibody, and the like. In some embodiments, the heterologous molecule is not a therapeutic agent. In some embodiments, the heterologous molecule does not exhibit a cytostatic or cytotoxic effect by itself.

[0054] The term "monoclonal antibody" refers to an antibody that is derived from a single cell clone, including any eukaryotic or prokaryotic cell clone, or a phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology.

[0055] The term "heterologous," in the context of a polypeptide, means from a different source (*e.g.*, a cell, tissue, organism, or species) as compared with another polypeptide, so that the two polypeptides are different. Typically, a heterologous polypeptide is from different species.

[0056] As used herein, the term "functional," in the context of an CD70 binding agent indicates that the binding agent is (1) capable of binding to CD70 and (2) depletes or inhibits the proliferation of CD70-expressing cells without conjugation to a cytotoxic or cytostatic agent, or has an immunsuppressive effect on an immune cell without conjugation to an immunosuppressive agent.

[0057] The term "antibody effector function(s)," or AEF, as used herein refers to a function contributed by an Fc effector domain(s) of an Ig (*e.g.*, the Fc region of an immunoglobulin). Such function can be effected by, for example, binding of an Fc effector domain(s) to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Typically, the effect(s) mediated by the Fc-binding cells or complement components result in inhibition and/or depletion of the CD70 targeted cell.

[0058] The term "antibody-dependent cellular cytotoxicity", or ADCC, is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells (*i.e.*, cells with bound antibody) with immune cells possessing lytic activity (also referred to as effector cells). Such effector cells include natural killer cells, monocytes/macrophages and neutrophils. ADCC is triggered by interactions between the Fc region of an antibody bound to a tumor cell and Fcγ receptors, particularly FcγRI and FcγRIII, on immune effector cells such as neutrophils, macrophages and natural killer cells. The tumor cell is eliminated by phagocytosis or lysis, depending upon the type of mediating effector cell. Death of the antibody-coated target cell occurs as a result of effector cell activity.

[0059] The term "antibody-dependent cellular phagocytosis", or ADCP, refers to the process by which antibody-coated cells are internalized, either in whole or in part, by phagocytic immune cells (*e.g.*, macrophages, neutrophils and dendritic cells) that bind to an immunoglobulin Fc region.

[0060] The term "complement-dependent cytotoxicity" or CDC refers to a mechanism for inducing cell death in which an Fc effector domain(s) of a target-bound antibody activates a series of enzymatic reactions culminating in the formation of holes in the target cell membrane. Typically, antigen-antibody complexes such as those on antibody-coated target cells bind and activate complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate ADCC by binding complement receptors (*e.g.*, CR3) on leukocytes.

[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

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same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence. To determine the percent identity, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions (*e.g.*, overlapping positions) x 100). In some embodiments, the two sequences are the same length.

[0062] The term "substantially identical," in the context of two nucleic acids or polypeptides, refers to two or more sequences or subsequences that have at least 50%, at least 55%, at least 60%, or at least 65% identity; typically at least 70% or at least 75% identity; more typically at least 80% or at least 85% identity; and even more typically at least 90%, at least 95%, or at least 98% identity (*e.g.*, as determined using one of the methods set forth *infra*).

[0063] The terms "similarity" or "percent similarity" in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of amino acid residues that are the same or conservatively substituted when compared and aligned for maximum correspondence, as measured using one of the methods set forth *infra*. By way of example, a first amino acid sequence can be considered similar to a second amino acid sequence when the first amino acid sequence is at least 50%, 60%, 70%, 75%, 80%, 90%, or 95% identical, or conservatively substituted, to the second amino acid sequence when compared to an equal number of amino acids as the number contained in the first sequence, or when compared to an alignment of polypeptides that has been aligned by a, *e.g.*, one of the methods set forth infra.

[0064] The terms "substantial similarity" or "substantially similar," in the context of polypeptide sequences, indicates that a polypeptide region has a sequence with at least 70%, typically at least 80%, more typically at least 85%, or at least 90% or at least 95% sequence

similarity to a reference sequence. For example, a polypeptide is substantially similar to a second polypeptide, when the two peptides differ by one or more conservative substitutions.

[0065] In the context of anti-CD70 antibodies or derivatives thereof, a protein that has one or more polypeptide regions substantially identical or substantially similar to one or more antigen-binding regions (*e.g.*, a heavy or light chain variable region, or a heavy or light chain CDR) of an anti-CD70 antibody retains specific binding to an epitope of CD70 recognized by the anti-CD70 antibody, as determined using any of various standard immunoassays known in the art or as referred to herein.

[0066] The determination of percent identity or percent similarity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid encoding a protein of interest. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein of interest. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci.USA* 85:2444-8. Within FASTA, ktup is a control option that sets the

sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA.

[0067] Alternatively, protein sequence alignment may be carried out using the CLUSTAL W algorithm, as described by Higgins *et al.*, 1996, *Methods Enzymol.* 266:383-402.

[0068] As used herein, the terms "prevention" or "prevent" refer to administration of an anti-CD70 antibody or derivative or other binding agent to a subject before the onset of a clinical or diagnostic symptom of a CD70-expressing cancer or immunological disorder (*e.g.*, administration to an individual with a predisposition or at a high risk of acquiring the CD70-expressing cancer or immunological disorder) to (a) block the occurrence or onset of the CD70-expressing cancer or immunological disorder, or one or more of clinical or diagnostic symptoms thereof, (b) inhibit the severity of onset of the CD70-expressing cancer or immunological disorder, or (c) to lessen the likelihood of the onset of the CD70-expressing cancer or immunological disorder.

[0069] As used herein, the terms "treatment" or "treat" refer to slowing, stopping, and/or reversing the progression of a CD70-expressing cancer or immunological disorder in a subject, as evidenced by a decrease or elimination of a clinical or diagnostic symptom of the disease, by administration of an anti-CD70 antibody or derivative thereof or other binding agent to the subject after the onset of the clinical or diagnostic symptom of the CD70-expressing cancer or immunological disorder at any clinical stage. Treatment can include, for example, a decrease in the severity of a symptom, the number of symptoms, or frequency of relapse.

[0070] The term "pharmaceutically acceptable" as used herein means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "pharmaceutically compatible ingredient" refers to a pharmaceutically acceptable diluent, adjuvant, excipient, or vehicle with which an anti-CD70-binding agent is administered.

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[0071] The term "effective amount" refers to the amount of the antibody or derivative or other binding agent that is sufficient to inhibit the occurrence or ameliorate one or more clinical or diagnostic symptoms of a CD70-expressing cancer or immunological disorder in a subject. An effective amount of an agent is administered according to the methods described herein in an "effective regime." The term "effective regime" refers to a combination of amount of the agent and dosage frequency adequate to accomplish treatment or prevention of a CD70-expressing cancer or immunological disorder.

II. Anti-CD70 Antibodies and Derivatives Thereof

[0072] The methods and compositions described herein encompass the use of a CD70 binding agent that specifically binds to CD70 and exerts a cytotoxic, cytostatic or immunosuppressive effect on CD70-expressing cancer cells or activated immune cells. The CD70 binding agent can be, for example, an anti-CD70 antibody, an antigen-binding fragment of an anti-CD70 antibody, a derivative thereof, or other CD70-binding agent. The CD70-binding agent includes an antibody effector domain function that mediates or stimulates ADCC, ADCP and/or CDC responses against a CD70-expressing target cell. The effector domain(s) can be, for example, an Fc region of an Ig molecule. The CD70-binding agent exerts a cytotoxic or cytostatic effect on CD70-expressing cancer cells, or exerts a cytotoxic, cytostatic, or immunosuppressive effect on activated lymphocytes or dendritic cells, for the treatment of a CD70-expressing cancer or an immunological disorder, respectively. Typically, the CD70-binding agent recruits and/or activates cytotoxic white blood cells (*e.g.*, natural killer (NK) cells, phagocytotic cells (*e.g.*, macrophages), and/or serum complement components). In some embodiments, the CD70 binding agent is monoclonal antibody (mAb) 1F6 or 2F2 or a derivative thereof. In other embodiments, the anti-CD70 antibody or derivative thereof competes with monoclonal antibody 1F6 or 2F2 for binding to CD70. In some embodiments, the CD70 binding agent does not induce an agonistic or antagonistic signal when binding to CD70.

[0073] An anti-CD70 antibody typically is or is derived from a monoclonal antibody and can include, for example, a chimeric (*e.g.*, having a human constant region and mouse variable region), a humanized, or a fully human antibody; a single chain antibody; a maxibody, a minibody, an antigen binding region, or the like. The antibody molecule includes at least one effector domain that can functionally interact with and activate cytotoxic white blood cells

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and/or serum complement components. In some embodiments, a CD7O antigen binding region can be joined to an effector domain or domains such as, for example, hinge-C_H2-C_H3 domains of an immunoglobulin, or a portion or fragment of an effector domain(s) having effector function. Antigen-binding antibody fragments, including single-chain antibodies, can comprise for example the variable region(s) in combination with the entirety or a portion of an effector domain (*e.g.*, a C_H2 and/or C_H3 domain alone or in combination with a C_H1, hinge and/or CL domain). Also, antigen-binding fragments can comprise any combination of effector domains. In some embodiments, the anti-CD70 antibody can be a single chain antibody comprising a CD70-binding variable region joined to hinge-C_H2-C_H3 domains.

[0074] Typically, the antibodies are of human, or non-human origin (*e.g.*, rodent (*e.g.*, mouse or rat)), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, or chicken) of specific Ig isotypes that can mediate effector function. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries, from human B cells, or from animals transgenic for one or more human immunoglobulin, as described *infra* and, for example in U.S. Patent Nos. 5,939,598 and 6,111,166.

[0075] The effector domain of an antibody can be from any suitable vertebrate animal species and isotypes. The isotypes from different animal species differ in the abilities to mediate effector functions. For example, the ability of human immunoglobulin to mediate CDC and ADCC/ADCP is generally in the order of IgM≈IgG1≈IgG3>IgG2>IgG4 and IgG1≈IgG3>IgG2/IgM/IgG4, respectively. Murine immunoglobulins mediate CDC and ADCC/ADCP generally in the order of murine IgM≈IgG3>>IgG2b>IgG2a>>IgG1 and IgG2b>IgGa>IgG1>>IgG3, respectively. In another example, murine IgG2a mediates ADCC while both murine IgG2a and IgM mediate CDC. In some embodiments, the CD70 binding agent consists of antibody variable and effector domains. In other embodiments, the CD70 binding agent consists essentially of antibody variable and effector domains, and can further include an additional compound(s) that is not a therapeutic agent(s). A CD70-binding polypeptide also can be expressed as a recombinant fusion protein comprising of the appropriate constant domains to yield the desired effector function(s).

[0076] Upon binding to target cells, the antibodies or derivatives can trigger *in vitro* and *in vivo* target cell destruction through effector domain (*e.g.*, Fc-) mediated effector functions.

Without intending to be bound by any particular theory, Fc regions of antibodies can recruit Fc receptor (FcR)-expressing cells and juxtapose them with antibody-coated target cells. Cells expressing surface FcR for IgGs including FcγRIII (CD16), FcγRII (CD32) and FcγRIII (CD64) can act as effector cells for the destruction of IgG-coated cells. Such effector cells include monocytes, macrophages, natural killer (NK) cells, neutrophils and eosinophils. Engagement of FcγR by IgG activates antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). ADCC is mediated by $CD16^+$ effector cells through the secretion of membrane pore-forming proteins and proteases, while phagocytosis is mediated by $CD32^+$ and $CD64^+$ effector cells (*see Fundamental Immunology*, 4th ed., Paul ed., Lippincott-Raven, N.Y., 1997, Chapters 3, 17 and 30; Uchida *et al.*, 2004, *J. Exp. Med.* 199:1659-69; Akewanlop *et al.*, 2001, *Cancer Res.* 61:4061-65; Watanabe *et al.*, 1999, *Breast Cancer Res. Treat.* 53:199-207). In addition to ADCC and ADCP, Fc regions of cell-bound antibodies can also activate the complement classical pathway to elicit complement-dependent cytotoxicity (CDC). C1q of the complement system binds to the Fc regions of antibodies when they are complexed with antigens. Binding of C1q to cell-bound antibodies can initiate a cascade of events involving the proteolytic activation of C4 and C2 to generate the C3 convertase. Cleavage of C3 to C3b by C3 convertase enables the activation of terminal complement components including C5b, C6, C7, C8 and C9. Collectively, these proteins form membrane-attack complex pores on the antibody-coated cells. These pores disrupt the cell membrane integrity, killing the target cell (see *Immunobiology*, 6th ed., Janeway *et al.*, Garland Science, N. Y., 2005, Chapter 2).

[0077] The antibodies can be monospecific, bispecific, trispecific, or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of CD70 and/or may be specific for both CD70 as well as for a heterologous protein. (*See, e.g.*, PCT Publications WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt *et al.*, 1991, *J. Immunol.* 147:60-69; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; and 5,601,819; Kostelny *et al.*, 1992, *J. Immunol.* 148:1547-1553.) Multispecific antibodies, including bispecific and trispecific antibodies, useful for practicing the methods described herein are antibodies that immunospecifically bind to both CD70 (including but not limited to antibodies that have the CDRs of the monoclonal antibodies 2F2 and 1F6) and a second cell surface receptor or receptor complex that mediates ADCC, phagocytosis, and/or CDC, such as CD16/FcgRIII, CD64/FcgRI, killer inhibitory or activating receptors, or the complement

control protein CD59. In a typical embodiment, the binding of the portion of the multispecific antibody to the second cell surface molecule or receptor complex enhances the effector functions of the anti-CD70 antibody or other CD70 binding agent.

[0078] In one aspect, an anti-CD70 antibody comprises one or more complementarity determining regions (CDRs) substantially identical or substantially similar to one or more CDR(s) of monoclonal antibody 1F6 (*see* Table 1). For example, the antibody can include a heavy chain CDR and/or a light chain CDR that is substantially identical or substantially similar to a corresponding heavy chain CDR (H1, H2, or H3 regions) or corresponding light chain CDR (L1, L2, or L3 regions) of mAb 1F6 (SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:16; SEQ ID NO:18; or SEQ ID NO:20, respectively). In typical embodiments, the anti-CD70 antibody has two or three heavy chain CDRs and/or two or three light chain CDRs that are substantially identical or substantially similar to corresponding heavy and/or light chain CDRs of mAb 1F6. In specific embodiments, a CDR substantially identical or substantially similar to a heavy or light chain CDR of 1F6 has the amino acid sequence set forth in SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:18, or SEQ ID NO:20.

[0079] For example, in some embodiments, where an anti-CD70 antibody has at least one heavy chain CDR substantially identical or substantially similar to a heavy chain CDR of mAb 1F6, the antibody or derivative thereof further includes at least one light chain CDR that is substantially identical or substantially similar to a light chain CDR of mAb 1F6.

[0080] In some embodiments, an anti-CD70 antibody includes a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs substantially identical or substantially similar to corresponding CDRs of mAb 1F6, and (b) a set of four framework regions. For example, an anti-CD70 antibody can include a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs, in which the set of CDRs are from monoclonal antibody 1F6, and (b) a set of four framework regions of the IgG type.

[0081] In some embodiments, the anti-CD70 antibody is a chimeric antibody. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as for example antibodies having a variable region derived from a murine monoclonal antibody and a human IgG immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. (*See e.g.*, Morrison, *Science*, 1985,

229:1202; Oi *et al.*, 1986, *BioTechniques* 4:214; Gillies *et al.*, 1989, *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397.)

[0082] In an exemplary embodiment, the anti-CD70 antibody is a chimeric antibody that includes a heavy chain variable region that is substantially identical or substantially similar to the heavy chain variable region of mAb 1F6 (*i.e.*, substantially identical or substantially similar to the amino acid sequences set forth in SEQ ID NO:2, *see* Table 1) and/or a light chain variable region that is substantially identical or substantially similar to the light chain variable regions of mAb 1F6 (*i.e.*, substantially identical or substantially similar to the amino acid sequences set forth in SEQ ID NO:12, *see* Table 1). For example, the antibody can include a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:2 and, optionally, can further include a light chain variable region having the amino acid sequence set forth in SEQ ID NO:12. The heavy and light chain antibody constant regions are of the IgG type. In an exemplary embodiment, the anti-CD70 antibody is a chimeric IgG mAb 1F6.

[0083] In some embodiments, an anti-CD70 antibody is a chimeric antibody that includes one or more CDRs substantially identical or substantially similar to one or more CDR(s) of monoclonal antibody 2F2 (*see* Table 1). For example, the antibody can include a heavy chain CDR and/or a light chain CDR that is substantially identical or substantially similar to a corresponding heavy chain CDR (H1, H2, or H3 regions) or corresponding light chain CDR (L1, L2, or L3 regions) of mAb 2F2 (SEQ ID NO:26, SEQ ID NO:28; SEQ ID NO:30; SEQ ID NO:36, SEQ ID NO:38 or SEQ ID NO:40). In typical embodiments, the anti-CD70 antibody has two or three heavy chain CDRs and/or two or three light chain CDRs that are substantially identical or substantially similar to corresponding heavy and/or light chain CDRs of mAb 2F2. In specific embodiments, a CDR substantially identical or substantially similar to a heavy or light chain CDR of 2F2 has the amino acid sequence set forth in SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30; SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO:40.

[0084] For example, in some embodiments, where an anti-CD70 antibody has at least one heavy chain CDR substantially identical or substantially similar to a heavy chain CDR of mAb 2F2, the antibody or derivative thereof further includes at least one light chain CDR that is substantially identical or substantially similar to a light chain CDR of mAb 2F2.

[0085] In some embodiments, an anti-CD70 antibody includes a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs substantially identical or substantially similar to corresponding CDRs of mAb 2F2, and (b) a set of four framework regions. For example, an anti-CD70 antibody can include a heavy or light chain variable domain, the variable domain having (a) a set of three CDRs, in which the set of CDRs are from monoclonal antibody 2F2, and (b) a set of four framework regions, in which the set of framework regions are of the IgG type. In an exemplary embodiment, the anti-CD70 antibody is a chimeric IgG mAb 2F2.

[0086] In an embodiment, the anti-CD70 antibody includes a heavy chain variable region that is substantially identical or substantially similar to the heavy chain variable region of mAb 2F2 (*i.e.*, substantially identical or substantially similar to the amino acid sequences set forth in SEQ ID NO:22, *see* Table 1) and/or a light chain variable region that is substantially identical or substantially similar to the light chain variable regions of mAb 2F2 (*i.e.*, substantially identical or substantially similar to the amino acid sequences set forth in SEQ ID NO:32, *see* Table 1). For example, the antibody can include a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO:22 and, optionally, can further include a light chain variable region having the amino acid sequence set forth in SEQ ID NO:32. In one exemplary embodiment, the anti-CD70 antibody is mAb 2F2.

[0087] In some embodiments, the antibody comprises a 1F6 VH and a 2F2 VL or a 1F6 VH and a 2F2 VL.

[0088] The following table indicates the regions of 1F6 or 2F2 to which each SEQ ID NO. corresponds.

Table 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
1F6 Heavy Chain Variable Region	Nucleotide	1
1F6 Heavy Chain Variable Region	Amino Acid	2

Table 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
1F6 Heavy Chain Signal Peptide	Nucleotide	3
1F6 Heavy Chain Signal Peptide	Amino Acid	4
1F6 Heavy Chain-CDR1(H1)	Nucleotide	5
1F6 Heavy Chain-CDR1(H1)	Amino Acid	6
1F6 Heavy Chain-CDR2(H2)	Nucleotide	7
1F6 Heavy Chain-CDR2(H2)	Amino Acid	8
1F6 Heavy Chain-CDR3(H3)	Nucleotide	9
1F6 Heavy Chain-CDR3(H3)	Amino Acid	10
1F6 Light Chain Variable Region	Nucleotide	11
1F6 Light Chain Variable Region	Amino Acid	12
1F6 Light Chain Signal Peptide	Nucleotide	13
1F6 Light Chain Signal Peptide	Amino Acid	14
1F6 Light Chain-CDR1(L1)	Nucleotide	15
1F6 Light Chain-CDR1(L1)	Amino Acid	16
1F6 Light Chain-CDR2(L2)	Nucleotide	17
1F6 Light Chain-CDR2(L2)	Amino Acid	18
1F6 Light Chain-CDR3(L3)	Nucleotide	19

Table 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
1F6 Light Chain-CDR3(L3)	Amino Acid	20
2F2 Heavy Chain Variable Region	Nucleotide	21
2F2 Heavy Chain Variable Region	Amino Acid	22
2F2 Heavy Chain Signal Peptide	Nucleotide	23
2F2 Heavy Chain Signal Peptide	Amino Acid	24
2F2 Heavy Chain-CDR1(H1)	Nucleotide	25
2F2 Heavy Chain-CDR1(H1)	Amino Acid	26
2F2 Heavy Chain-CDR2(H2)	Nucleotide	27
2F2 Heavy Chain-CDR2(H2)	Amino Acid	28
2F2 Heavy Chain-CDR3(H3)	Nucleotide	29
2F2 Heavy Chain-CDR3(H3)	Amino Acid	30
2F2 Light Chain Variable Region	Nucleotide	31
2F2 Light Chain Variable Region	Amino Acid	32
2F2 Light Chain Signal Peptide	Nucleotide	33
2F2 Light Chain Signal Peptide	Amino Acid	34
2F2 Light Chain-CDR1(L1)	Nucleotide	35
2F2 Light Chain-CDR1(L1)	Amino Acid	36

Table 1

MOLECULE	NUCLEOTIDE OR AMINO ACID	SEQ ID NO
2F2 Light Chain-CDR2(L2)	Nucleotide	37
2F2 Light Chain-CDR2(L2)	Amino Acid	38
2F2 Light Chain-CDR3(L3)	Nucleotide	39
2F2 Light Chain-CDR3(L3)	Amino Acid	40

[0089] Anti-CD70 antibodies and derivatives thereof and other binding agents may also be described or specified in terms of their binding affinity to CD70. Typical binding affinities include those with a dissociation constant or Kd less than 5 X 10^{-2} M, 10^{-2} M, 5 X 10^{-3} M, 10^{-3} M, 5 X 10^{-4} M, 10^{-4} M, 5 X 10^{-5} M, 10^{-5} M, 5 X 10^{-6} M, 10^{-6} M, 5 X 10^{-7} M, 10^{-7} M, 5 X 10^{-8} M, 10^{-8} M, 5 X 10^{-9} M, 10^{-9} M, 5 X 10^{-10} M, 10^{-10} M, 5 X 10^{-11} M, 10^{-11} M, 5 X 10^{-12} M, 10^{-12} M, 5 X $^{-13}$ M, 10^{-13} M, 5 X 10^{-14} M, 10^{-14} M, 5 X 10^{-15} M, or 10^{-15} M.

[0090] The antibodies can be generated by methods known in the art. For example, monoclonal antibodies can be prepared using a wide variety of techniques including, *e.g.*, the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. Hybridoma techniques are generally discussed in, for example, Harlow *et al.*, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); and Hammerling, *et al.*, In *Monoclonal Antibodies and T-Cell Hybridomas*, pp. 563-681 (Elsevier, N.Y., 1981). Examples of phage display methods that can be used to make the anti-CD70 antibodies include, *e.g.*, those disclosed in (Hoogenboom and Winter, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581; Quan and Carter, 2002, *The rise of monoclonal antibodies as therapeutics* in *Anti-IgE and Allergic Disease*, Jardieu and Fick Jr., eds., Marcel Dekker, New York, NY, Chapter 20, pp. 427–469; Brinkman et al., 1995, *J. Immunol. Methods* 182:41-50; Ames *et al.*, 1995, *J. Immunol. Methods* 184:177-186; Kettleborough *et al.*, 1994, *Eur. J. Immunol.* 24:952-958; Persic *et al.*, 1997, *Gene* 187:9-18; Burton *et al.*, 1994, *Advances in Immunology* 57:191-280; PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/11236,

WO 95/15982, WO 95/20401, and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

[0091] Examples of techniques that can be used to produce single-chain antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al.*, 1991, *Methods in Enzymology* 203:46-88; Shu *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:7995-7999; and Skerra *et al.*, 1988, *Science* 240:1038-1040.

[0092] Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (*see, e.g.*, Milstein *et al.*, 1983, Nature 305:537-39). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Similar procedures are disclosed in International Publication No. WO 93/08829, and in Traunecker *et al.*, 1991, EMBO J. 10:3655-59.

[0093] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. In some embodiments, the fusion includes a first heavy-chain constant region (C_H1) containing the site necessary for light chain binding, present in at least one of the fusions. Nucleic acids with sequences encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0094] In an embodiment of this approach, the bispecific antibodies have a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation (*see*, *e.g.*, International Publication No. WO 94/04690.

[0095] For further discussion of bispecific antibodies see, for example, Suresh *et al.*, 1986, *Methods in Enzymology* 121:210; Rodrigues *et al.*, 1993, *J. Immunology* 151:6954-61; Carter *et al.*, 1992, *Bio/Technology* 10:163-67; Carter *et al.*, 1995, *J. Hematotherapy* 4:463-70; Merchant *et al.*, 1998, *Nature Biotechnology* 16:677-81. Using such techniques, bispecific antibodies can be prepared for use in the treatment or prevention of disease as defined herein.

[0096] Bifunctional antibodies are also described in European Patent Publication No. EPA 0 105 360. As disclosed in this reference, hybrid or bifunctional antibodies can be derived either biologically, *i.e.*, by cell fusion techniques, or chemically, especially with cross-linking agents or disulfide-bridge forming reagents, and may comprise whole antibodies or fragments thereof. Methods for obtaining such hybrid antibodies are disclosed for example, in International Publication WO 83/03679 and European Patent Publication No. EPA 0 217 577.

[0097] An anti-CD70 antibody can also be a humanized antibody. Humanized antibodies are antibody molecules that bind the desired antigen and have one or more CDRs from a non-human species, and framework and constant regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See*, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, 1988, *Nature* 332:323.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (*see*, *e.g.*, EP 0 239 400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (*see*, *e.g.*, EP 0 592 106; EP

0 519 596; Padlan, *Molecular Immunology*, 1991, 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:969-973), and chain shuffling (*see, e.g.*, U.S. Patent No. 5,565,332).

[0098] Humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Publication No. WO 87/02671; European Patent Publication No. 0 184 187; European Patent Publication No. 0 171 496; European Patent Publication No. 0 173 494; International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Publication No. 0 012 023; Berter *et al.*, 1988, *Science* 240:1041-43; Liu *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-43; Liu *et al.*, 1987, *J. Immunol.* 139:3521-26; Sun *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:214-18; Nishimura *et al.*, 1987, *Cancer. Res.* 47:999-1005; Wood *et al.*, 1985, *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl. Cancer Inst.* 80:1553-59; Morrison, 1985, *Science* 229:1202-07; Oi *et al.*, 1986, *BioTechniques* 4:214; U.S. Patent 5,225,539; Jones *et al.*, 1986, *Nature* 321:552-25; Verhoeyan *et al.*, 1988, *Science* 239:1534; and Beidler *et al.*, 1988, *J. Immunol.* 141:4053-60.

[0099] In some embodiments, the anti-CD70 antibody is a human IgG antibody. Human antibodies can be made by a variety of methods known in the art including, *e.g.*, phage display methods (*see supra*) using antibody libraries derived from human immunoglobulin sequences. *See also, e.g.*, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT Publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741. In addition, a human antibody recognizing a selected epitope can be generated using a technique referred to as "guided selection," in which a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (*see, e.g.*, Jespers *et al.*, 1994, *Bio/technology* 12:899-903). Human antibodies can also be produced using transgenic mice that express human immunoglobulin genes. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. For an overview of this technology for producing human antibodies, *see* Lonberg and Huszar, 1995, *Int. Rev. Immunol.* 13:65-93. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, PCT Publications WO 98/24893, WO 92/01047, WO 96/34096, and WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos.

5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598.

[0100] In addition, companies such as Abgenix, Inc. (Fremont, CA), Genpharm (San Jose, CA), and Medarex (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above. Completely human antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies. (*See, e.g.*, U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806.

[0101] As set forth *supra*, a CD70 binding agent can be a derivative of an anti-CD70 antibody. Generally, an anti-CD70 antibody derivative comprises an anti-CD70 antibody (including *e.g.*, an antigen-binding fragment or conservatively substituted polypeptides) and at least one polypeptide region or other moiety heterologous to the anti-CD70 antibody. For example, an anti-CD70 antibody can be modified, *e.g.*, by the covalent attachment of any type of molecule, such that covalent attachment does not prevent the antibody derivative from specifically binding to CD70 via the antigen-binding region or region derived therefrom, or the effector domains(s) from specifically binding Fc receptor. Typical modifications include,

e.g., glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, and the like. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.*

[0102] In some embodiments, the antibody derivative is a multimer, such as, for example, a dimer, comprising one or more monomers, where each monomer includes (i) an antigen-binding region of an anti-CD70 antibody, or a polypeptide region derived therefrom (such as, *e.g.*, by conservative substitution of one or more amino acids), and (ii) a multimerizing (*e.g.*, dimerizing) polypeptide region, such that the antibody derivative forms multimers (*e.g.*, homodimers) that specifically bind to CD70. In typical embodiments, an antigen binding region of an anti-CD70 antibody, or a polypeptide region derived therefrom, is recombinantly or chemically fused with a heterologous protein, wherein the heterologous protein comprises a dimerization or multimerization domain. Prior to administration of the antibody derivative to a subject for the purpose of treating or preventing immunological disorders or CD70-expressing cancers, the derivative is subjected to conditions that allow formation of a homodimer or heterodimer. A heterodimer, as used herein, may comprise identical dimerization domains but different CD70 antigen-binding regions, identical CD70 antigen-binding regions but different dimerization domains, or different CD70 antigen-binding regions and dimerization domains.

[0103] Typical dimerization domains are those that originate from transcription factors. In one embodiment, the dimerization domain is that of a basic region leucine zipper ("bZIP") (*see* Vinson *et al.*, 1989, *Science* 246:911-916). Useful leucine zipper domains include, for example, those of the yeast transcription factor GCN4, the mammalian transcription factor CCAAT/enhancer-binding protein C/EBP, and the nuclear transform in oncogene products, Fos and Jun. (*See* Landschultz *et al.*, 1988, *Science* 240:1759-64; Baxevanis and Vinson, 1993, *Curr. Op. Gen. Devel.* 3:278-285; O'Shea *et al.*, 1989, *Science* 243:538-542.) In another embodiment, the dimerization domain is that of a basic-region helix-loop-helix ("bHLH") protein. (*See* Murre *et al.*, 1989, *Cell* 56:777-783. *See also* Davis *et al.*, 1990, *Cell* 60:733-746; Voronova and Baltimore, 1990, *Proc. Natl. Acad. Sci. USA* 87:4722-26.) Particularly useful hHLH proteins are myc, max, and mac.

[0104] In yet other embodiments, the dimerization domain is an immunoglobulin constant region such as, for example, a heavy chain constant region or a domain thereof (*e.g.*, a C_H1 domain, a C_H2 domain, and/or a C_H3 domain). (*See, e.g.*, U.S. Patent Nos. 5,155,027; 5,336,603; 5,359,046; and 5,349,053; EP 0 367 166; WO 96/04388.)

[0105] Heterodimers are known to form between Fos and Jun (Bohmann *et al.*, 1987, *Science* 238:1386-1392), among members of the ATF/CREB family (Hai *et al.*, 1989, *Genes Dev.* 3:2083-2090), among members of the C/EBP family (Cao *et al.*, 1991, *Genes Dev.* 5:1538-52; Williams *et al.*, 1991, *Genes Dev.* 5:1553-67; Roman *et al.*, 1990, *Genes Dev.* 4:1404-15), and between members of the ATF/CREB and Fos/Jun families (Hai and Curran, 1991, *Proc. Natl. Acad. Sci. USA* 88:3720-24). Therefore, when a CD70-binding protein is administered to a subject as a heterodimer comprising different dimerization domains, any combination of the foregoing may be used.

[0106] In other embodiments, an anti-CD70 antibody derivative is an anti-CD70 antibody conjugated to a second antibody (an "antibody heteroconjugate") (*see* U.S. Patent No. 4,676,980). Heteroconjugates useful for practicing the present methods comprise an antibody that binds to CD70 (*e.g.*, an antibody that has the CDRs and/or heavy chains of the monoclonal antibodies 2F2 or 1F6) and an antibody that binds to a surface receptor or receptor complex, such as CD16/FcgRIII, CD64/FcgRI, killer cell activating or inhibitory receptors, or the complement control protein CD59. In a typical embodiment, the binding of the portion of the multispecific antibody to the second cell surface molecule or receptor complex enhances the effector functions of an anti-CD70 antibody.

[0107] In some embodiments, the anti-CD70 antibody or derivative thereof competitively inhibits binding of mAb 1F6 or 2F2 to CD70, as determined by any method known in the art for determining competitive binding (such as *e.g.*, the immunoassays described herein). In typical embodiments, the antibody competitively inhibits binding of 1F6 or 2F2 to CD70 by at least 50%, at least 60%, at least 70%, or at least 75%. In other embodiments, the antibody competitively inhibits binding of 1F6 or 2F2 to CD70 by at least 80%, at least 85%, at least 90%, or at least 95%.

[0108] Antibodies can be assayed for specific binding to CD70 by any of various known methods. Immunoassays which can be used include, for example, competitive and non-competitive assay systems using techniques such as Western blots, radioimmunoassays,

ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well-known in the art. (*See, e.g.*, Ausubel *et al.*, eds., *Short Protocols in Molecular Biology* (John Wiley and Sons, Inc., New York, 4th ed. 1999); Harlow and Lane, *Using Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999.)

[0109] Further, the binding affinity of an antibody to CD70 and the off-rate of an antibody CD70 interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled CD70 (*e.g.*, ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled CD70, and the detection of the antibody bound to the labeled CD70. The affinity of the antibody for CD70 and the binding off-rates can then be determined from the data by Scatchard plot analysis. Competition with a second antibody (such as *e.g.*, mAb 1F6 or 2F2) can also be determined using radioimmunoassays. For example, CD70 is incubated with the antibody of interest conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody. Alternatively, the binding affinity of an antibody to CD70 and the on- and off-rates of an antibody-CD70 interaction can be determined by surface plasmon resonance. In some embodiments, the anti-CD70 antibodies or derivatives thereof can be targeted to and accumulate on the membrane of a CD70-expressing cell.

[0110] The anti-CD70 antibodies and derivatives thereof that are useful in the present methods can be produced by methods known in the art for the synthesis of proteins, typically, *e.g.*, by recombinant expression techniques. Recombinant expression of an antibody or derivative thereof that binds to CD70 and depletes or inhibits the proliferation of CD70-expressing cells requires construction of an expression vector containing a nucleic acid that encodes the antibody or derivative thereof. A vector for the production of the protein molecule may be produced by recombinant DNA technology using techniques known in the art. Standard techniques such as, for example, those described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 3rd ed., 2001); Sambrook *et al.*, *Molecular Cloning: A Laboratory*

Manual (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2nd ed., 1989); *Short Protocols in Molecular Biology* (Ausubel *et al.*, John Wiley and Sons, New York, 4th ed., 1999); and Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (ASM Press, Washington, D.C., 2nd ed., 1998) can be used for recombinant nucleic acid methods, nucleic acid synthesis, cell culture, transgene incorporation, and recombinant protein expression.

[0111] For example, for recombinant expression of an anti-CD70 antibody, an expression vector may encode a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. An expression vector may include, for example, the nucleotide sequence encoding the constant region of the antibody molecule (*see, e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464), and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain. The expression vector is transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the anti-CD70 antibody. In typical embodiments for the expression of double-chain antibodies, vectors encoding both the heavy and light chains can be co-expressed in the host cell for expression of the entire immunoglobulin molecule.

[0112] A variety of prokaryotic and eukaryotic host-expression vector systems can be utilized to express an anti-CD70 antibody or derivative thereof. Typically, eukaryotic cells, particularly for whole recombinant anti-CD70 antibody molecules, are used for the expression of the recombinant protein. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus, is an effective expression system for the production of anti-CD70 antibodies and derivatives thereof (*see, e.g.*, Foecking *et al.*, 1986, *Gene* 45:101; Cockett *et al.*, 1990, *Bio/Technology* 8:2).

[0113] Other host-expression systems include, for example, plasmid-based expression systems in bacterial cells (*see, e.g.*, Ruther *et al.*, 1983, *EMBO* 1,2:1791; Inouye and Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke and Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); insect systems such as, *e.g.*, the use of *Autographa californica* nuclear polyhedrosis virus (AcNPV) expression vector in *Spodoptera frugiperda* cells; and viral-based expression systems in mammalian cells, such as, *e.g.*, adenoviral-based systems (*see*,

e.g., Logan and Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359; Bittner *et al.*, 1987, *Methods in Enzymol.* 153:51-544).

[0114] In addition, a host cell strain can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing (*e.g.*, glycosylation, phosphorylation, and cleavage) of the protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript and gene product can be used. Such mammalian host cells include, for example, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and W138.

[0115] A stable expression system is typically used for long-term, high-yield production of recombinant anti-CD70 antibody or derivative thereof. For example, cell lines that stably express the anti-CD70 antibody or derivative thereof can be engineered by transformation of host cells with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites) and a selectable marker, followed by growth of the transformed cells in a selective media. The selectable marker confers resistance to the selection and allows cells to stably integrate the DNA into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. A number of selection systems can be used, including, for example, the herpes simplex virus thymidine kinase, hypoxanthineguanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, which can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate; gpt, which confers resistance to mycophenolic acid; neo, which confers resistance to the aminoglycoside G-418; and hygro, which confers resistance to hygromycin. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone, and such methods are described, for example, in *Current Protocols in Molecular Biology* (Ausubel *et al.* eds., John Wiley and Sons, N.Y., 1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual* (Stockton Press, N.Y., 1990); *Current Protocols in Human Genetics* (Dracopoli *et al.* eds., John Wiley and Sons, N.Y., 1994, Chapters 12 and 13); and Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1.

[0116] The expression levels of an antibody or derivative can be increased by vector amplification. (*See generally, e.g.*, Bebbington and Hentschel, *The Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning, Vol.3* (Academic Press, New York, 1987).) When a marker in the vector system expressing an anti-CD70 antibody or derivative thereof is amplifiable, an increase in the level of inhibitor present in host cell culture media will select host cells that have increased copy number of a marker gene conferring resistance to the inhibitor. The copy number of an associated antibody gene will also be increased, thereby increasing expression of the antibody or derivative thereof (*see* Crouse *et al.*, 1983, *Mol. Cell. Biol.* 3:257).

[0117] Where the anti-CD70 antibody comprises both a heavy and a light chain or derivatives thereof, the host cell may be co-transfected with two expression vectors, the first vector encoding the heavy chain protein and the second vector encoding the light chain protein. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain proteins. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain proteins. In such situations, the light chain is typically placed before the heavy chain to avoid an excess of toxic free heavy chain (*see* Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0118] Once an anti-CD70 antibody or derivative thereof has has been produced (*e.g.*, by an animal, chemical synthesis, or recombinant expression), it can be purified by any suitable method for purification of proteins, including, for example, by chromatography (*e.g.*, ion exchange or affinity chromatography (such as, for example, Protein A chromatography for purification of antibodies having an intact Fc region)), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. An anti-CD70 antibody or derivative thereof can, for example, be fused to a marker sequence, such as a peptide, to facilitate purification by affinity chromatography. Suitable marker amino acid sequences include, *e.g.*, a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA, 91311), and the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell* 37:767), and the "flag" tag.

[0119] Once an anti-CD70 antibody or derivative thereof is produced, its ability to exert a cytostatic or cytotoxic effect on CD70-expressing cancer cells or an immunosuppressive effect on a CD70-expressing immune cell is determined by the methods described *infra* or as known in the art.

[0120] To minimize activity of the anti-CD70 antibody outside the activated immune cells or CD70-expressing cancer cells, an antibody that specifically binds to cell membrane-bound CD70, but not to soluble CD70, can be used, so that the anti-CD70 antibody is concentrated at the cell surface of the activated immune cell or CD70-expressing cancer cell.

[0121] Typically, the anti-CD70 antibody or derivative is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). In some embodiments, the anti-CD70 antibody or derivative is at least about 40% pure, at least about 50% pure, or at least about 60% pure. In some embodiments, the anti-CD70 antibody or derivative is at least about 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, or 95-98% pure. In some embodiments, the anti-CD70 antibody or derivative is approximately 99% pure.

III. Other CD70-Binding Agent

[0122] Further CD70-binding agents include fusion proteins (*i.e.*, proteins that are recombinantly fused or chemically conjugated, including both covalent and non-covalent conjugation) to heterologous proteins (of typically at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 amino acids). Such CD70-binding agents include a portion that binds to CD70 and an immunoglobulin effector domain or a functional equivalent thereof. As used herein, a functional equivalent of immunoglobulin effector domain binds to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. The fusion protein does not necessarily need to be direct, but may occur through linker sequences.

[0123] For example, CD70-binding agent can be produced recombinantly by fusing the coding region of one or more of the CDRs of an anti-CD70 antibody in frame with a sequence coding for a heterologous protein. The heterologous protein includes an effector domain or a functional equivalent thereof and may provide one or more of the following

characteristics: promote stable expression; provide a means of facilitating high yield recombinant expression; and/or provide a multimerization domain.

[0124] In some embodiments, the CD70-binding agent can include one or more CDRs from an antibody that binds to CD70 and depletes or inhibits the proliferation of CD70-expressing cells alone, without conjugation to a cytotoxic agent.

[0125] In an aspect, a CD70-binding agent can include CD27 and variants or fragments thereof that bind to CD70. CD70-binding agent can further include peptides, ligands and other molecules that specifically bind to CD70.

[0126] CD70-binding agent can be identified using any method suitable for screening for protein-protein interactions. Typically, proteins are initially identified by their ability to specifically bind to CD70. The ability of such a binding protein to exert a cytostatic or cytotoxic effect on activated lymphocytes or CD70-expressing cancer cells by recruiting and activating cytotoxic white blood cells, *e.g.*, natural killer (NK) cells, phagocytotic cells, *e.g.*, macrophages, and serum complement components, without conjugation to a cytotoxic or cytostatic agent, or an immunosuppressive effect on an immune cell by themselves, without conjugation to an immunosuppressive agent, can be determined. Among the traditional methods which can be employed are "interaction cloning" techniques which entail probing expression libraries with labeled CD70 in a manner similar to the technique of antibody probing of λgt11 libraries. By way of example and not limitation, this can be achieved as follows: a cDNA clone encoding CD70 (or a 1F6 or 2F2 binding domain thereof) can be modified at the C-terminus by inserting the phosphorylation site for the heart muscle kinase (HMK) (*see, e.g.*, Blanar and Rutter, 1992, *Science* 256:1014-18). The recombinant protein is expressed in *E. coli* and purified on a GDP-affinity column to homogeneity (Edery *et al.*, 1988, *Gene* 74:517-25) and labeled using γ^{32}P-ATP and bovine heart muscle kinase (Sigma) to a specific activity of $1x10^8$ cpm/μg, and used to screen a human placenta λgt11 cDNA library in a "far-Western assay" (Blanar and Rutter, 1992, *Science* 256:1014-18). Plaques which interact with the CD70 probe are isolated. The cDNA inserts of positive λ plaques are released and subcloned into a vector suitable for sequencing, such as pBluescript KS (Stratagene, La Jolla, CA).

[0127] One method which detects protein interactions *in vivo* is the two-hybrid system. One version of this system has been described (Chien *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:9578-82) and is commercially available from Clontech (Palo Alto, CA).

[0128] Once a CD70-binding protein is identified, its ability (alone or when multimerized or fused to a dimerization or multimerization domain) to exert a cytostatic or cytotoxic effect on CD70-expressing cancer cells or an immunosuppressive effect on a CD70-expressing immune cell can be determined by the methods described *infra*.

IV. Methods to improve effector functions of anti-CD70-targeting agents.

[0129] In some embodiments, the effector function of a CD70-binding agent can be augmented by improving its effector functions using one or more antibody engineering approaches known in the art. Illustrative, non-limiting examples for such approaches are provided below.

[0130] ADCC and ADCP are mediated through the interaction of cell-bound antibodies with Fcγ receptors (FcγR) expressed on effector cells. Both the glycosylation status and primary amino acid sequence of the IgG Fc region have functional effects on the Fcγ-FcγR interaction. A stronger Fcγ-FcγR interaction is associated with better target cell killing by effector cells.

[0131] Oligosaccharides covalently attached to the conserved Asn297 are required for the Fc region of an IgG to bind FcγR (Lund *et al.*, 1996, *J. Immunol.* 157:4963-69; Wright and Morrison, 1997, *Trends Biotechnol.* 15:26-31). Engineering of this glycoform on IgG can significantly improve IgG-mediated ADCC. Addition of bisecting N-acetylglucosamine modifications (Umana *et al.,* 1999, *Nat. Biotechnol.* 17:176-180; Davies *et al.,* 2001, *Biotech. Bioeng.* 74:288-94) to this glycoform or removal of fucose (Shields *et al.,* 2002, *J. Biol. Chem.* 277:26733-40; Shinkawa *et al.,* 2003, *J. Biol. Chem.* 278:6591-604; Niwa *et al.,* 2004, *Cancer Res.* 64:2127-33) from this glycoform are two examples of IgG Fc engineering that improves the binding between IgG Fc and FcγR, thereby enhancing Ig-mediated ADCC activity.

[0132] A systemic substitution of solvent-exposed amino acids of human IgG1 Fc region has generated IgG variants with altered FcγR binding affinities (Shields *et al.,* 2001, *J. Biol.*

Chem. 276:6591-604). When compared to parental IgG1, a subset of these variants involving substitutions at Thr256/Ser298, Ser298/Glu333, Ser298/Lys334, or Ser298/Glu333/Lys334 to Ala demonstrate increased in both binding affinity toward FcγR and ADCC activity (Shields *et al.*, 2001, *J. Biol. Chem.* 276:6591-604; Okazaki *et al.*, 2004, *J. Mol. Biol.* 336:1239-49).

[0133] Antibody-mediated CDC begins with the binding of C1q to cell bound IgG molecules. Specific amino acid residues on human IgG1 responsible for C1q binding and species-specific differences of C1q binding have been reported (Idusogie *et al.*, 2000, *J. Immunol.* 164:4178-4184). Complement fixation activity of antibodies have been improved by substitutions at Lys326 and Glu333; for *e.g.*, such substitutions can improve both C1q binding and CDC activity of the human IgG1 antibody rituximab (Idusogie *et al.*, 2001, *J. Immunol.* 166:2571-2575). The same substitutions on a human IgG2 backbone can convert an antibody isotype that binds poorly to C1q and is severely deficient in complement activation activity to one that can both bind C1q and mediate CDC (Idusogie *et al.*, 2001, *J. Immunol.* 166:2571-75). Several other methods have also been applied to improve complement fixation activity of antibodies. For example, the grafting of an 18-amino acid carboxyl-terminal tail piece of IgM to the carboxyl-termini of IgG greatly enhances their CDC activity. This is observed even with IgG4, which normally has no detectable CDC activity (Smith *et al.*, 1995, *J. Immunol.* 154:2226-36). Also, substituting Ser444 located close to the carboxy-terminal of IgG1 heavy chain with Cys induced tail-to-tail dimerization of IgG1 with a 200-fold increase of CDC activity over monomeric IgG1 (Shopes *et al.*, 1992, *J. Immunol.* 148:2918-22). In addition, a bispecific diabody construct with specificity for C1q also confers CDC activity (Kontermann *et al.*, 1997, *Nat. Biotech.* 15:629-31).

[0134] The *in vivo* half-life of an antibody can also impact on its effector functions. In some embodiments, it is desirable to increase or decrease the half-life of an antibody to modify its therapeutic activities. FcRn is a receptor that is structurally similar to MHC Class I antigen that non-covalently associates with β2-microglobulin. FcRn regulates the catabolism of IgGs and their transcytosis across tissues (Ghetie and Ward, 2000, *Annu. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The IgG-FcRn interaction takes place at pH 6.0 (pH of intracellular vesicles) but not at pH 7.4 (pH of blood); this interaction enables IgGs to be recycled back to the circulation (Ghetie and Ward, 2000, *Ann. Rev. Immunol.* 18:739-766; Ghetie and Ward, 2002, *Immunol. Res.* 25:97-113). The region on human IgG1 involved in FcRn binding has been mapped (Shields *et al.*, 2001,

J. Biol. Chem. 276:6591-604). Alanine substitutions at positions Pro238, Thr256, Thr307, Gln311, Asp312, Glu380, Glu382, or Asn434 of human IgG1 enhance FcRn binding (Shields *et al.,* 2001, *J. Biol. Chem.* 276:6591-604). IgG1 molecules harboring these substitutions are expected to have longer serum half-lives. Consequently, these modified IgG1 molecules may be able to carry out their effector functions, and hence exert their therapeutic efficacies, over a longer period of time compared to unmodified IgG1.

V. Assays for Cytotoxic, Cytostatic, and Immunosuppressive Activities

[0135] Methods of determining whether an antibody mediates effector function against a target cell are known. Illustrative examples of such methods are described *infra.*

[0136] For determining whether an anti-CD70 antibody or derivative mediates antibody-dependent cellular cytotoxicity against activated immune cells or CD70-expressing cancer cells, an assay that measures target cell death in the presence of antibody and effector immune cells may be used. An assay used to measure this type of cytotoxicity can be based on determination of ^{51}Cr release from metabolically-labeled targets cells after incubation in the presence of effector cells and target-specific antibody (*see, e.g.,* Perussia and Loza, 2000, *Methods in Molecular Biology* 121:179-92 and "^{51}Cr Release Assay of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)," in *Current Potocols in Immunology*, Coligan *et al.* eds., Wileyand Sons, 1993). For example, activated immune cells (*e.g.*, activated lymphocytes) or CD70-expressing cancer cells labeled with $Na_2{}^{51}CrO_4$ and plated at a density of 5,000 cells per well of a 96-well plate can be treated with varying concentrations of anti-CD70 antibody for 30 minutes and then mixed with normal human peripheral blood mononuclear cells (PBMC) for 4 hours. The membrane disruption that accompanies target cell death releases ^{51}Cr into the culture supernatant which may be collected and assessed for radioactivity as a measure of cytotoxic activity. Other assays to measure ADCC may involve nonradioactive labels or be based on induced release of specific enzymes. For example, a non-radioactive assay based on time-resolved fluorometry is commercially available (Delphia, Perkin Elmer). This assay is based on loading target cells with an acetoxymethyl ester of fluorescence enhancing ligand (BATDA) that penetrates the cell membrane then hydrolyses to form a membrane impermeable hydrophilic ligand (TDA). When mixed with target specific antibody and PBMC effector cells, TDA is released from lysed cells and is

available to form a highly fluorescent chelate when mixed with Europium. The signal, measured with a time-resolved fluorometer, correlates with the amount of cell lysis.

[0137] To determine whether an anti-CD70 antibody or derivative mediates antibody-dependent cellular phagocytosis against activated immune cells or CD70-expressing cancer cells, an assay that measures target cell internalization by effector immune cells (*e.g.*, fresh cultured macrophages or established macrophage-like cell line) may be used (*see*, *e.g.*, Munn and Cheung, 1990, *J. Exp. Med.* 172:231-37; Keler *et al.*, 2000, *J. Immunol.* 164:5746-52; Akewanlop *et al.*, 2001, *Cancer Res.* 61:4061-65). For example, target cells may be labeled with a lipophilic membrane dye such as PKH67 (Sigma), coated with target-specific antibody, and mixed with effector immune cells for 4-24 hours. The effector cells may then be identified by counterstaining with a fluorochrome-labeled antibody specific for a phagocytic cell surface marker (*e.g.*, CD14) and the cells analyzed by two-color flow cytometry or fluoresence microscopy. Dual-positive cells represent effector cells that have internalized target cells. For these assays, effector cells may be monocytes derived from PBMC that have been differentiated into macrophages by culture for 5-10 days with M-CSF or GM-CSF (*see*, *e.g.*, Munn and Cheung, *supra*). Human macrophage-like cell lines U937 (Larrick *et al.*, 1980, *J. Immunology* 125:6-12) or THP-1 (Tsuchiya *et al.*, 1980, *Int. J. Cancer* 26:171-76) which are available from ATCC may be used as an alternative phagocytic cell source.

[0138] Methods of determining whether an antibody mediates complement-dependent cytotoxicity upon binding to target cells are also known. The same methods can be applied to determine whether a CD70-binding agent mediates CDC activated immune cells or CD70-expressing cancer cells. Illustrative examples of such methods are described *infra*.

[0139] The source of active complements can either be normal human serum or purified from laboratory animal including rabbits. In a standard assay, a CD70-binding agent is incubated with CD70-expressing activated immune cells (*e.g.*, activated lymphocytes) or CD70-expressing cancer cells in the presence of complements. The ability of such CD70-binding agent to mediate cell lysis can be determined by several readouts. In one example, a $Na^{51}CrO_4$ release assay is used. In this assay, target cells are labeled with $Na^{51}CrO_4$. Unincorporated $Na^{51}CrO_4$ is washed off and cells are plated at a suitable density, typically between 5,000 to 50,000 cells/well, in a 96-well plate. Incubation with the CD70-binding

agent in the presence of normal serum or purified complements typically last for 2-6 hours at 37°C in a 5% CO_2 atmosphere. Released radioactivity, indicating cell lysis, is determined in an aliquot of the culture supernatant by gamma ray counting. Maximum cell lysis is determined by releasing incorporated $Na^{51}CrO_4$ by detergent (0.5-1% NP-40 or Triton X-100) treatment. Spontaneous background cell lysis is determined in wells where only complements are present without any CD70-binding agents. Percentage cell lysis is calculated as (CD70-binding agent-induced lysis – spontaneous lysis)/maximum cell lysis. The second readout is a reduction of metabolic dyes, *e.g.*, Alamar Blue, by viable cells. In this assay, target cells are incubated with CD70-binding agent with complements and incubated as described above. At the end of incubation, 1/10 volume of Alamar Blue (Biosource International, Camarillo, CA) is added. Incubation is continued for up to 16 hours at 37°C in a 5% CO_2 atmosphere. Reduction of Alamar Blue as an indication of metabolically active viable cells is determined by fluorometric analysis with excitation at 530 nm and emission at 590 nm. The third readout is cellular membrane permeability to propidium iodide (PI). Formation of pores in the plasma membrane as a result of complement activation facilitates entry of PI into cells where it will diffuse into the nuclei and bind DNA. Upon binding to DNA, PI fluorescence in the 600 nm significantly increases. Treatment of target cells with CD70-binding agent and complements is carried out as described above. At end of incubation, PI is added to a final concentration of 5 μg/ml. The cell suspension is then examined by flow cytometry using a 488 nm argon laser for excitation. Lysed cells are detected by fluorescence emission at 600 nm.

VI. Animal Models of Immunological Disorders or CD70-Expressing Cancers

[0140] The anti-CD70 antibodies or derivatives can be tested or validated in animal models of immunological disorders or CD70-expressing cancers. A number of established animal models of immunological disorders or CD70-expressing cancers are known to the skilled artisan, any of which can be used to assay the efficacy of the anti-CD70 antibody or derivative. Non-limiting examples of such models are described *infra*.

[0141] Examples for animal models of systemic and organ-specific autoimmune diseases including diabetes, lupus, systemic sclerosis, Sjögren's Syndrome, experimental autoimmune encephalomyelitis (multiple sclerosis), thyroiditis, myasthenia gravis, arthritis, uveitis, inflammatory bowel disease have been described by Bigazzi, "Animal Models of

Autoimmunity: Spontaneous and Induced," in *The Autoimmune Diseases* (Rose and Mackay eds., Academic Press, 1998); in "Animal Models for Autoimmune and Inflammatory Disease," in *Current Protocols in Immunology* (Coligan *et al.* eds., Wiley and Sons, 1997); and Peng, "Experimental Use of Murine Lupus Models," in *Methods in Molecular Medicine*, Vol. 102. *Autoimmunity: Methods and Protocols* (Perl ed., Humana Press Inc.).

[0142] Allergic conditions, *e.g.*, asthma and dermatitis, can also be modeled in rodents. Airway hypersensitivity can be induced in mice by ovalbumin (Tomkinson *et al.*, 2001, *J. Immunol.* 166:5792-800) or *Schistosoma mansoni* egg antigen (Tesciuba *et al.*, 2001, *J. Immunol.* 167:1996-2003). The Nc/Nga strain of mice show marked increase in serum IgE and spontaneously develop atopic dermatitis-like leisons (Vestergaard *et al.*, 2000, *Mol. Med. Today* 6:209-10; Watanabe *et al.*, 1997, *Int. Immunol.* 9:461-66; Saskawa *et al.*, 2001, *Int. Arch. Allergy Immunol.* 126:239-47).

[0143] Injection of immuno-competent donor lymphocytes into a lethally irradiated histo-incompatible host is a classical approach to induce GVHD in mice. Alternatively, the parent B6D2F1 murine model provides a system to induce both acute and chronic GVHD. In this model the B6D2F1 mice are F1 progeny from a cross between the parental strains of C57BL/6 and DBA/2 mice. Transfer of DBA/2 lymphoid cells into non-irradiated B6D2F1 mice causes chronic GVHD, whereas transfer of C57BL/6, C57BL/10 or B10.D2 lymphoid cells causes acute GVHD (Slayback *et al.*, 2000, *Bone Marrow Transpl.* 26:931-938; Kataoka *et al.*, 2001, *Immunology* 103:310-318).

[0144] Additionally, both human hematopoietic stem cells and mature peripheral blood lymphoid cells can be engrafted into SCID mice, and these human lympho-hematopoietic cells remain functional in the SCID mice (McCune *et al.*, 1988, *Science* 241:1632-1639; Kamel-Reid and Dick, 1988, *Science* 242:1706-1709; Mosier *et al.*, 1988, *Nature* 335:256-259). This has provided a small animal model system for the direct testing of potential therapeutic agents on human lymphoid cells. (*See, e.g.*, Tournoy *et al.*, 2001, *J. Immunol.* 166:6982-6991.)

[0145] Moreover, small animal models to examine the *in vivo* efficacies of the anti-CD70 antibodies or derivatives can be created by implanting CD70-expressing human tumor cell lines into appropriate immunodeficient rodent strains, *e.g.*, athymic nude mice or SCID mice. Examples of CD70-expressing human lymphoma cell lines include, for example, Daudi

(Ghetie *et al.*, 1994, *Blood* 83:1329-36; Ghetie *et al.*, 1990, *Int. J. Cancer* 15:481-85; de Mont *et al.*, 2001, *Cancer Res.* 61:7654-59), Ramos (Ma *et al.*, 2002, *Leukemia* 16:60-6; Press *et al.*, 2001, *Blood* 98:2535-43), HS-Sultan (Cattan and Maung, 1996, *Cancer Chemother. Pharmacol.* 38:548-52; Cattan and Douglas, 1994, *Leuk. Res.* 18:513-22), Raji (Ochakovskaya *et al.*, 2001, *Clin. Cancer Res.* 7:1505-10; Breisto *et al.*, 1999, *Cancer Res.* 59:2944-49), and CA46 (Kreitman *et al.*, 1999, *Int. J. Cancer* 81:148-55). Non-limiting example of a CD70-expressing Hodgkin's lymphoma line is L540cy (Barth *et al.*, 2000, *Blood* 95:3909-14; Wahl *et al.*, 2002, *Cancer Res.* 62:3736-42). Non-limiting examples of CD70 expressing human renal cell carcinoma cell lines include 786-O (Ananth *et al.*, 1999, *Cancer Res.* 59:2210-16; Datta *et al.*, 2001, *Cancer Res.* 61:1768-75), ACHN (Hara *et al.*, 2001, *J. Urol.* 166:2491-94; Miyake *et al.*, 2002, *J. Urol.* 167:2203-08), Caki-1 (Prewett *et al.*, 1998, *Clin. Cancer Res.* 4:2957-66; Shi and Siemann, 2002, *Br. J. Cancer* 87:119-26), and Caki-2 (Zellweger *et al.*, 2001, *Neoplasia* 3:360-67). Non-limiting examples of CD70-expressing nasopharyngeal carcinoma cell lines include C15 and C17 (Busson *et al.*, 1988, *Int. J. Cancer* 42:599-606; Bernheim *et al.*, 1993, *Cancer Genet. Cytogenet.* 66:11-5). Non-limiting examples of CD70-expressing human glioma cell lines include U373 (Palma *et al.*, 2000, *Br. J. Cancer* 82:480-7) and U87MG (Johns *et al.*, 2002, *Int. J. Cancer* 98:398-408). These tumor cell lines can be established in immunodeficient rodent hosts either as solid tumor by subcutaneous injections or as disseminated tumors by intravenous injections. Once established within a host, these tumor models can be applied to evaluate the therapeutic efficacies of the anti-CD70 antibody or derivatives as described herein on modulating *in vivo* tumor growth.

VII. Immune Disorders and CD70-expressing Cancers

[0146] The anti-CD70 antibodies or derivatives as described herein are useful for treating or preventing a CD70-expressing cancer or an immunological disorder characterized by expression of CD70 by inappropriate activation of immune cells (*e.g.*, lymphocytes or dendritic cells). Such expression of CD70 can be due to, for example, increased CD70 protein levels on the cells surface and/or altered antigenicity of the expressed CD70. Treatment or prevention of the immunological disorder, according to the methods described herein, is achieved by administering to a subject in need of such treatment or prevention an effective amount of the anti-CD70 antibody or derivative, whereby the antibody or derivative (i) binds to activated immune cells that express CD70 and that are associated with the disease

state and (ii) exerts a cytotoxic, cytostatic, or immunosuppressive effect on the activated immune cells without conjugation to a cytotoxic, cytostatic, or immunosuppressive agent.

[0147] Immunological diseases that are characterized by inappropriate activation of immune cells and that can be treated or prevented by the methods described herein can be classified, for example, by the type(s) of hypersensitivity reaction(s) that underlie the disorder. These reactions are typically classified into four types: anaphylactic reactions, cytotoxic (cytolytic) reactions, immune complex reactions, or cell-mediated immunity (CMI) reactions (also referred to as delayed-type hypersensitivity (DTH) reactions). (*See, e.g., Fundamental Immunology* (William E. Paul ed., Raven Press, N.Y., 3rd ed. 1993).)

[0148] Specific examples of such immunological diseases include the following: rheumatoid arthritis, autoimmune demyelinative diseases (*e.g.*, multiple sclerosis, allergic encephalomyelitis), endocrine ophthalmopathy, uveoretinitis, systemic lupus erythematosus, myasthenia gravis, Grave's disease, glomerulonephritis, autoimmune hepatological disorder, inflammatory bowel disease (*e.g.*, Crohn's disease), anaphylaxis, allergic reaction, Sjogren's syndrome, type I diabetes mellitus, primary biliary cirrhosis, Wegener's granulomatosis, fibromyalgia, polymyositis, dermatomyositis, multiple endocrine failure, Schmidt's syndrome, autoimmune uveitis, Addison's disease, adrenalitis, thyroiditis, Hashimoto's thyroiditis, autoimmune thyroid disease, pernicious anemia, gastric atrophy, chronic hepatitis, lupoid hepatitis, atherosclerosis, subacute cutaneous lupus erythematosus, hypoparathyroidism, Dressler's syndrome, autoimmune thrombocytopenia, idiopathic thrombocytopenic purpura, hemolytic anemia, pemphigus vulgaris, pemphigus, dermatitis herpetiformis, alopecia arcata, pemphigoid, scleroderma, progressive systemic sclerosis, CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), male and female autoimmune infertility, ankylosing spondolytis, ulcerative colitis, mixed connective tissue disease, polyarteritis nedosa, systemic necrotizing vasculitis, atopic dermatitis, atopic rhinitis, Goodpasture's syndrome, Chagas' disease, sarcoidosis, rheumatic fever, asthma, recurrent abortion, anti-phospholipid syndrome, farmer's lung, erythema multiforme, post cardiotomy syndrome, Cushing's syndrome, autoimmune chronic active hepatitis, bird-fancier's lung, toxic epidermal necrolysis, Alport's syndrome, alveolitis, allergic alveolitis, fibrosing alveolitis, interstitial lung disease, erythema nodosum, pyoderma gangrenosum, transfusion reaction, Takayasu's arteritis, polymyalgia rheumatica, temporal arteritis, schistosomiasis, giant cell arteritis,

ascariasis, aspergillosis, Sampter's syndrome, eczema, lymphomatoid granulomatosis, Behcet's disease, Caplan's syndrome, Kawasaki's disease, dengue, encephalomyelitis, endocarditis, endomyocardial fibrosis, endophthalmitis, erythema elevatum et diutinum, psoriasis, erythroblastosis fetalis, eosinophilic faciitis, Shulman's syndrome, Felty's syndrome, filariasis, cyclitis, chronic cyclitis, heterochronic cyclitis, Fuch's cyclitis, IgA nephropathy, Henoch-Schonlein purpura, graft versus host disease, transplantation rejection, cardiomyopathy, Eaton-Lambert syndrome, relapsing polychondritis, cryoglobulinemia, Waldenstrom's macroglobulemia, Evan's syndrome, and autoimmune gonadal failure.

[0149] Accordingly, the methods described herein encompass treatment of disorders of B lymphocytes (*e.g.*, systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes), Th_1-lymphocytes (*e.g.*, rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, tuberculosis, or graft versus host disease), or Th_2-lymphocytes (*e.g.*, atopic dermatitis, systemic lupus erythematosus, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, or chronic graft versus host disease). Generally, disorders involving dendritic cells involve disorders of Th_1-lymphocytes or Th_2-lymphocytes.

[0150] In some embodiments, the immunological disorder is a T cell-mediated immunological disorder, such as a T cell disorder in which activated T cells associated with the disorder express CD70. Anti-CD70 antibodies or derivatives can be administered to deplete such CD70-expressing activated T cells. In a specific embodiment, administration of anti-CD70 antibodies or derivatives can deplete CD70-expressing activated T cells, while resting T cells are not substantially depleted by the anti-CD70 or derivative. In this context, "not substantially depleted" means that less than about 60%, or less than about 70% or less than about 80% of resting T cells are not depleted.

[0151] The anti-CD70 antibodies and derivatives as described herein are also useful for treating or preventing a CD70-expressing cancer. Treatment or prevention of a CD70-expressing cancer, according to the methods described herein, is achieved by administering to a subject in need of such treatment or prevention an effective amount of the anti-CD70 antibody or derivative, whereby the antibody or derivative (i) binds to CD70-expressing cancer cells and (ii) exerts a cytotoxic or cytostatic effect to deplete or inhibit the

proliferation of the CD70-expressing cancer cells alone (*i.e.*, without conjugation to a cytotoxic, cytostatic, or immunosuppressive agent).

[0152] CD70-expressing cancers that can be treated or prevented by the methods described herein include, for example, different subtypes of indolent Non-Hodgkin's Lymphoma (indolent NHLs) (*e.g.*, follicular NHLs, small lymphocytic lymphomas, lymphoplasmacytic NHLs, or marginal zone NHLs); Non-Hodgkin's Lymphoma, cancers of the B-cell lineage, including, *e.g.*, Burkitt's lymphoma and chronic lymphocytic leukemia; multiple myeloma, renal cell carcinomas; nasopharyngeal carcinomas; thymic carcinomas; gliomas; glioblastoma, Waldenströms macroglobulinemia; meningiomas; and colon, stomach, and rectal carcinomas.

VIII. Pharmaceutical Compositions Comprising Anti-CD70 Antibodies and Derivatives and Administration Thereof

[0153] A composition containing a CD70 binding agent (*e.g.*, an anti-CD70 antibody or derivative) can be administered to a subject having or at risk of having an immunological disorder or a CD70-expressing cancer. The invention further provides for the use of a CD70 binding agent (*e.g.*, an anti-CD70 antibody or derivative) in the manufacture of a medicament for prevention or treatment of a CD70 expressing cancer or immunological disorder. The term "subject" as used herein means any mammalian patient to which a CD70-binding agent can be administered, including, *e.g.*, humans and non-human mammals, such as primates, rodents, and dogs. Subjects specifically intended for treatment using the methods described herein include humans. The antibodies or derivatives can be administered either alone or in combination with other compositions in the prevention or treatment of the immunological disorder or CD70-expressing cancer.

[0154] Various delivery systems are known and can be used to administer the CD70 binding agent. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The CD70 binding agent can be administered, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, and the like) and can be administered together with other biologically active agents such as chemotherapeutic agents. Administration can be systemic or local.

[0155] In specific embodiments, the CD70 binding agent composition is administered by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber. Typically, when administering the composition, materials to which the anti-CD70 antibody or derivative does not absorb are used.

[0156] In other embodiments, the anti-CD70 antibody or derivative is delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, 1990, *Science* 249:1527-1533; Sefton, 1989, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald *et al.*, 1980, *Surgery* 88:507; Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used. (*See Medical Applications of Controlled Release* (Langer and Wise eds., CRC Press, Boca Raton, Florida, 1974); *Controlled Drug Bioavailability, Drug Product Design and Performance* (Smolen and Ball eds., Wiley, New York, 1984); Ranger and Peppas, 1983, *Macromol. Sci. Rev. Macromol. Chem.* 23:61. *See also* Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 71:105.) Other controlled release systems are discussed, for example, in Langer, *supra.*

[0157] A CD70 binding agent (*e.g.*, an anti-CD70 antibody or derivative) can be administered as pharmaceutical compositions comprising a therapeutically effective amount of the binding agent and one or more pharmaceutically compatible ingredients. For example, the pharmaceutical composition typically includes one or more pharmaceutical carriers (*e.g.*, sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like). Water is a more typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard

carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the nucleic acid or protein, typically in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulations correspond to the mode of administration.

[0158] In typical embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical can also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[0159] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a CD70 binding agent (*e.g.*, an anti-CD70 antibody or derivative) in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (*e.g.*, sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the lyophilized anti-CD70 antibody or derivative. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0160] The amount of the CD70 binding agent (*e.g.*, anti-CD70 antibody or derivative) that is effective in the treatment or prevention of an immunological disorder or CD70-expressing cancer can be determined by standard clinical techniques. In addition, *in vitro* assays may

optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the stage of immunological disorder or CD70-expressing cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0161] For example, toxicity and therapeutic efficacy of the anti-CD70 antibody or derivative can be determined in cell cultures or experimental animals by standard pharmaceutical procedures for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. A CD70-binding agent (*e.g.*, an anti-CD70 antibody or derivative) that exhibits a large therapeutic index is preferred. Where a CD70-binding agent exhibits toxic side effects, a delivery system that targets the CD70-binding agent to the site of affected tissue can be used to minimize potential damage non-CD70-expressing cells and, thereby, reduce side effects.

[0162] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of the CD70 binding agent typically lies within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any CD70 binding agent used in the method, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0163] Generally, the dosage of an anti-CD70 antibody or derivative administered to a patient with an immunological disorder or CD70-expressing cancer is typically 0.1 mg/kg to 100 mg/kg of the subject's body weight. The dosage administered to a subject is 0.1 mg/kg to 50 mg/kg of the subject's body weight, 1 mg/kg to 30 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 15 mg/kg, or 1 mg/kg to 10 mg/kg of the subject's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species

due to the immune response to the foreign proteins. Thus, lower dosages of anti-CD70 antibody or derivative comprising humanized, chimeric or human antibodies and less frequent administration is often possible.

[0164] In some embodiments, the pharmaceutical compositions comprising the CD70 binding agent can further comprise a therapeutic agent (*i.e.*, a non-conjugated cytotoxic or immunosuppressive agent such as, for example, any of those described herein). The anti-CD70 binding agent also can be co-administered in combination with one or more therapeutic agents for the treatment or prevention of immunological disorders or CD70-expressing cancers. For example, combination therapy can include a therapeutic agent (*e.g.*, a cytostatic, cytotoxic, or immunosuppressive agent, such as an unconjugated cytostatic, cytotoxic, or immunosuppressive agent such as those conventionally used for the treatment of cancers or immunological disorders). Combination therapy can also include, *e.g.*, administration of an agent that targets a receptor or receptor complex other than CD70 on the surface of activated lymphocytes, dendritic cells or CD70-expressing cancer cells. An example of such an agent includes a second, non-CD70 antibody that binds to a molecule at the surface of an activated lymphocyte, dendritic cell or CD70-expressing cancer cell. Another example includes a ligand that targets such a receptor or receptor complex. Typically, such an antibody or ligand binds to a cell surface receptor on activated lymphocytes, dendritic cell or CD70-expressing cancer cell and enhances the cytotoxic or cytostatic effect of the anti-CD70 antibody by delivering a cytostatic or cytotoxic signal to the activated lymphocyte, dendritic cell or CD70-expressing cancer cell. Such combinatorial administration can have an additive or synergistic effect on disease parameters (*e.g.*, severity of a symptom, the number of symptoms, or frequency of relapse).

[0165] With respect to therapeutic regimens for combinatorial administration, in a specific embodiment, an anti-CD70 binding agent is administered concurrently with a therapeutic agent. In another specific embodiment, the therapeutic agent is administered prior or subsequent to administration of the anti-CD70 antibody or derivative, by at least an hour and up to several months, for example at least an hour, five hours, 12 hours, a day, a week, a month, or three months, prior or subsequent to administration of the anti-CD70 antibody or derivative. In some embodiments, the subject is monitored following administration of the anti-CD70 binding agent, and optionally the therapeutic agent.

[0166] The therapeutic agent can be, for example, any agent that exerts a therapeutic effect on cancer cells or activated immune cells. Typically, the therapeutic agent is a cytotoxic or immunosuppressive agent.

[0167] Useful classes of cytotoxic or immunosuppressive agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (*e.g.*, platinum complexes such as *cis*-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like.

[0168] Individual cytotoxic or immunosuppressive agents include, for example, an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (formerly actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluordeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbizine, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

[0169] In some typical embodiments, the therapeutic agent is a cytotoxic agent. Suitable cytotoxic agents include, for example, dolastatins (*e.g.*, auristatin E, AFP, MMAF, MMAE), DNA minor groove binders (*e.g.*, enediynes and lexitropsins), duocarmycins, taxanes (*e.g.*, paclitaxel and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemadotin, maytansinoids, discodermolide, eleutherobin, and mitoxantrone.

[0170] In some embodiments, the cytotoxic agent is a conventional chemotherapeutic such as, for example, doxorubicin, paclitaxel, melphalan, vinca alkaloids, methotrexate, mitomycin

C or etoposide. In addition, potent agents such as CC-1065 analogues, calicheamicin, maytansine, analogues of dolastatin 10, rhizoxin, and palytoxin can be linked to the anti-CD70 antibodies or derivatives thereof.

[0171] In specific embodiments, the cytotoxic or cytostatic agent is auristatin E (also known in the art as dolastatin-10) or a derivative thereof. Typically, the auristatin E derivative is, *e.g.*, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatin derivatives include AFP, MMAF, and MMAE. The synthesis and structure of auristatin E and its derivatives are described in U.S. Patent Application Nos. 09/845,786 (U.S. Patent Application Publication No. 20030083263) and 10/001,191; International Patent Application No. PCT/US03/24209, International Patent Application No. PCT/US02/13435, and U.S. Patent Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414.

[0172] In specific embodiments, the cytotoxic agent is a DNA minor groove binding agent. (*See, e.g.*, U.S. Patent No. 6,130,237.) For example, in some embodiments, the minor groove binding agent is a CBI compound. In other embodiments, the minor groove binding agent is an enediyne (*e.g.*, calicheamicin).

[0173] Examples of anti-tubulin agents include, but are not limited to, taxanes (*e.g.*, Taxol® (paclitaxel), Taxotere® (docetaxel)), T67 (Tularik), vinca alkyloids (*e.g.*, vincristine, vinblastine, vindesine, and vinorelbine), and dolastatins (*e.g.*, auristatin E, AFP, MMAF, MMAE, AEB, AEVB). Other antitubulin agents include, for example, baccatin derivatives, taxane analogs (*e.g.*, epothilone A and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermolide, and eleutherobin.

[0174] In some embodiments, the cytotoxic agent is a maytansinoid, another group of anti-tubulin agents. For example, in specific embodiments, the maytansinoid is maytansine or DM-1 (ImmunoGen, Inc.; *see also* Chari *et al.*, 1992, *Cancer Res.* 52:127-131).

[0175] In some embodiments, the therapeutic agent is not a radioisotope.

[0176] In some embodiments, the cytotoxic or immunosuppressive agent is an antimetabolite. The antimetabolite can be, for example, a purine antagonist (*e.g.* azothioprine or mycophenolate mofetil), a dihydrofolate reductase inhibitor (*e.g.*, methotrexate), acyclovir, gangcyclovir, zidovudine, vidarabine, ribavarin, azidothymidine, cytidine arabinoside, amantadine, dideoxyuridine, iododeoxyuridine, poscarnet, or trifluridine.

[0177] In other embodiments, the cytotoxic or immunosuppressive agent is tacrolimus, cyclosporine or rapamycin. In further embodiments, the cytoxic agent is aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, bexarotene, bexarotene, calusterone, capecitabine, celecoxib, cladribine, Darbepoetin alfa, Denileukin diftitox, dexrazoxane, dromostanolone propionate, epirubicin, Epoetin alfa, estramustine, exemestane, Filgrastim, floxuridine, fludarabine, fulvestrant, gemcitabine, gemtuzumab ozogamicin, goserelin, idarubicin, ifosfamide, imatinib mesylate, Interferon alfa-2a, irinotecan, letrozole, leucovorin, levamisole, meclorethamine or nitrogen mustard, megestrol, mesna, methotrexate, methoxsalen, mitomycin C, mitotane, nandrolone phenpropionate, oprelvekin, oxaliplatin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pentostatin, pipobroman, plicamycin, porfimer sodium, procarbazine, quinacrine, rasburicase, Sargramostim, streptozocin, tamoxifen, temozolomide, teniposide, testolactone, thioguanine, toremifene, Tositumomab, Trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine and zoledronate.

[0178] In additional embodiments, the therapeutic agent is an antibody, such as a humanized anti HER2 monoclonal antibody, RITUXAN (rituximab; Genentech; a chimeric anti CD20 monoclonal antibody); OVAREX (AltaRex Corporation, MA); PANOREX (Glaxo Wellcome, NC; a murine IgG2a antibody); Cetuximab Erbitux (Imclone Systems Inc., NY; an anti-EGFR IgG chimeric antibody); Vitaxin (MedImmune, Inc., MD; Campath I/H (Leukosite, MA; a humanized IgG1 antibody); Smart MI95 (Protein Design Labs, Inc., CA; a humanized anti-CD33 IgG antibody); LymphoCide (Immunomedics, Inc., NJ; a humanized anti-CD22 IgG antibody); Smart ID10 (Protein Design Labs, Inc., CA; a humanized anti-HLA-DR antibody); Oncolym (Techniclone, Inc., CA; a radiolabeled murine anti-HLA-Dr10 antibody); Allomune (BioTransplant, CA; a humanized anti-CD2 mAb); Avastin (Genentech, Inc., CA; an anti-VEGF humanized antibody); Epratuzamab (Immunomedics, Inc., NJ and Amgen, CA; an anti-CD22 antibody); and CEAcide (Immunomedics, NJ; a humanized anti-CEA antibody).

[0179] Other suitable antibodies include, but are not limited to, antibodies against the following antigens: CA125, CA15-3, CA19-9, L6, Lewis Y, Lewis X, alpha fetoprotein, CA 242, placental alkaline phosphatase, prostate specific membrane antigen, prostatic acid phosphatase, epidermal growth factor, MAGE-1, MAGE-2, MAGE-3, MAGE -4, anti-transferrin receptor, p97, MUC1-KLH, CEA, gp100, MART1, Prostate Specific Antigen, IL-2 receptor, CD20, CD52, CD33, CD22, human chorionic gonadotropin, CD38, CD40, mucin, P21, MPG, and Neu oncogene product.

[0180] In some embodiments, the therapeutic agent is an immunosuppressive agent. The immunosuppressive agent can be, for example, gancyclovir, etanercept, tacrolimus, cyclosporine, rapamycin, cyclophosphamide, azathioprine, mycophenolate mofetil or methotrexate. Alternatively, the immunosuppressive agent can be, for example, a glucocorticoid (*e.g.*, cortisol or aldosterone) or a glucocorticoid analogue (*e.g.*, prednisone or dexamethasone).

[0181] In some typical embodiments, the immunosuppressive agent is an anti-inflammatory agent, such as arylcarboxylic derivatives, pyrazole-containing derivatives, oxicam derivatives and nicotinic acid derivatives. Classes of anti-inflammatory agents include, for example, cyclooxygenase inhibitors, 5-lipoxygenase inhibitors, and leukotriene receptor antagonists.

[0182] Suitable cyclooxygenase inhibitors include meclofenamic acid, mefenamic acid, carprofen, diclofenac, diflunisal, fenbufen, fenoprofen, ibuprofen, indomethacin, ketoprofen, nabumetone, naproxen, sulindac, tenoxicam, tolmetin, and acetylsalicylic acid.

[0183] Suitable lipoxygenase inhibitors include redox inhibitors (*e.g.*, catechol butane derivatives, nordihydroguaiaretic acid (NDGA), masoprocol, phenidone, Ianopalen, indazolinones, naphazatrom, benzofuranol, alkylhydroxylamine), and non-redox inhibitors (*e.g.*, hydroxythiazoles, methoxyalkylthiazoles, benzopyrans and derivatives thereof, methoxytetrahydropyran, boswellic acids and acetylated derivatives of boswellic acids, and quinolinemethoxyphenylacetic acids substituted with cycloalkyl radicals), and precursors of redox inhibitors.

[0184] Other suitable lipoxygenase inhibitors include antioxidants (*e.g.*, phenols, propyl gallate, flavonoids and/or naturally occurring substrates containing flavonoids, hydroxylated derivatives of the flavones, flavonol, dihydroquercetin, luteolin, galangin, orobol, derivatives

of chalcone, 4,2',4'-trihydroxychalcone, ortho-aminophenols, N-hydroxyureas, benzofuranols, ebselen and species that increase the activity of the reducing selenoenzymes), iron chelating agents (*e.g.*, hydroxamic acids and derivatives thereof, N-hydroxyureas, 2-benzyl-1-naphthol, catechols, hydroxylamines, carnosol trolox C, catechol, naphthol, sulfasalazine, zyleuton, 5-hydroxyanthranilic acid and 4-(omega-arylalkyl)phenylalkanoic acids), imidazole-containing compounds (*e.g.*, ketoconazole and itraconazole), phenothiazines, and benzopyran derivatives.

[0185] Yet other suitable lipoxygenase inhibitors include inhibitors of eicosanoids (*e.g.*, octadecatetraenoic, eicosatetraenoic, docosapentaenoic, eicosahexaenoic and docosahexaenoic acids and esters thereof, PGE1 (prostaglandin E1), PGA2 (prostaglandin A2), viprostol, 15-monohydroxyeicosatetraenoic, 15-monohydroxy-eicosatrienoic and 15-monohydroxyeicosapentaenoic acids, and leukotrienes B5, C5 and D5), compounds interfering with calcium flows, phenothiazines, diphenylbutylamines, verapamil, fuscoside, curcumin, chlorogenic acid, caffeic acid, 5,8,11,14-eicosatetrayenoic acid (ETYA), hydroxyphenylretinamide, Ionapalen, esculin, diethylcarbamazine, phenantroline, baicalein, proxicromil, thioethers, diallyl sulfide and di-(1-propenyl) sulfide.

[0186] Leukotriene receptor antagonists include calcitriol, ontazolast, Bayer Bay-x-1005, Ciba-Geigy CGS-25019C, ebselen, Leo Denmark ETH-615, Lilly LY-293111, Ono ONO-4057, Terumo TMK-688, Boehringer Ingleheim BI-RM-270, Lilly LY 213024, Lilly LY 264086, Lilly LY 292728, Ono ONO LB457, Pfizer 105696, Perdue Frederick PF 10042, Rhone-Poulenc Rorer RP 66153, SmithKline Beecham SB-201146, SmithKline Beecham SB-201993, SmithKline Beecham SB-209247, Searle SC-53228, Sumitamo SM 15178, American Home Products Way 121006, Bayer Bay-o-8276, Warner-Lambert CI-987, Warner-Lambert CI-987BPC-15LY 223982, Lilly LY 233569, Lilly LY-255283, MacroNex MNX-160, Merck and Co. MK-591, Merck and Co. MK-886, Ono ONO-LB-448, Purdue Frederick PF-5901, Rhone-Poulenc Rorer RG 14893, Rhone-Poulenc Rorer RP 66364, Rhone-Poulenc Rorer RP 69698, Shionoogi S-2474, Searle SC-41930, Searle SC-50505, Searle SC-51146, Searle SC-52798, SmithKline Beecham SKandF-104493, Leo Denmark SR-2566, Tanabe T-757 and Teijin TEI-1338.

[0187] *Examples*

[0188] The invention is further described in the following examples, which are in not intended to limit the scope of the invention. Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC) or Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ). Cell culture reagents were obtained from Invitrogen Corp., Carlsbad, CA.

[0189] *Example 1. Construction of Chimeric AntiCD70 Antibody.*

[0190] To determine the cDNA sequences encoding the light (VL) and heavy (VH) chain variable regions of 1F6 and 2F2 mAb, total RNA was isolated from the 1F6 and 2F2 hybridomas using TRIzol® Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Gene-specific primers mIgcK1: 5'-CTT CCA CTT GAC ATT GAT GTC TTT G-3' (SEQ ID NO:41) and mIgG1: 5'-CAG GTC ACT GTC ACT GGC TCA G-3' (SEQ ID NO:42) were applied to reverse transcribe the light chain variable (VL) and heavy chain variable (VH) first strand cDNAs from both RNA preparations, respectively. First strand cDNA reactions were run using the SuperScript™ First Strand Synthesis System for RT-PCR from Invitrogen. The V_L and V_H cDNAs were then poly-G tailed using terminal deoxynucleotidyl transferase (TdT) and the supplied TdT buffer, according to conditions specified by the manufacturer (Invitrogen). Poly-G tailed V_L and V_H first strand cDNAs were then subjected to PCR amplification. The forward primer for both the V_L and V_H PCRs was ANCTAIL: 5' GTC GAT GAG CTC TAG AAT TCG TGC CCC CCC CCC CCC C-3' (SEQ ID NO:43). The reverse primer for amplifying the V_L was HBS-mck: 5'-CGT CAT GTC GAC GGA TCC AAG CTT CAA GAA GCA CAC GAC TGA GGC AC-3' (SEQ ID NO:44). The reverse primer for amplifying the V_H was HBS-mG1: 5'-CGT CAT GTC GAC GGA TCC AAG CTT GTC ACC ATG GAG TTA GTT TGG GC-3' (SEQ ID NO:45). PCRs were run with Ex Taq and the supplied reaction buffer in conditions specified by the manufacturer (Fisher Scientific, Pittsburgh, PA). The V_L and V_H PCR products were then cut by HindIII and EcoRI and cloned into HindIII/EcoRI-cut pUC19. Recombinant plasmid clones were identified, and the nucleotide sequences for the 1F6 and 2F2 hybridomas were determined.

[0191] Complementarity determining regions (CDRs) in the heavy and light chains of 1F6 and 2F2 mAbs were determined according to the criteria described in Kabat *et al.*, 1991,

Sequences of Proteins of Immunological Interest, Washington DC, US Department of Health and Public Services; Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-17 (Figures 1 and 2). Sequence alignments at both the cDNA and amino acid levels revealed that closely related light chain genes were probably utilized in both hybridomas. There is a 92% sequence identity between 1F6 V_L and 2F2 V_L at the amino acid level. Sequence comparison of the CDRs shows that 1F6 CDR-L1 is identical to 2F2 CDR-L1, only one divergent substitution is present between 1F6 CDR-L2 and 2F2 CDR-L2, and only 2 conservative substitutions are present between 1F6 CDR-L3 and 2F2 CDR-L3 (Figure 3). On the other hand, a higher degree of sequence diversity is present between 1F6 V_H and 2F2 V_H; about 66 of 137 amino acid residues are different between the 2 V_Hs. Sequence comparison of the CDRs shows that 5 of 10 residues are different between 1F6 CDR-H1 and 2F2 CDR-H1 (3 of the 5 substitutions are divergent), 12 of 17 residues are different between 1F6 CDR-H2 and 2F2 CDR-H2 (9 of the 12 substitutions are divergent), and 5 of 9 residues are different between 1F6 CDR-H3 and 2F2 CDR-H3 (4 of the 5 substitutions are divergent) (Figure 3).

[0192] An expression vector containing both the chimeric 1F6 heavy and light chains was constructed, as described *infra*. In this vector, each of the polypeptide chains is under the control of a copy of the CHEF1 promoter, along with an associated CHEF1 intron sequence and an immunoglobulin polyA region. For construction of this large vector, it was necessary to assemble the chimerized heavy and light chain sequences in separate "chimerization" plasmids, each encoding a section of the final construct. The final expression construct was then assembled via three-way ligation with a third vector encoding the rest of the necessary control regions. DG44 CHO cells were transformed with this plasmid and a clonal line with good production was isolated.

[0193] *Construction of the heavy chain chimerization vector*

[0194] A chimerization vector, pSG850, had been previously constructed by cloning a 6 kb NotI-XhoI fragment of a CHEF1 vector into a Bluescript vector. This fragment contains part of the CHEF1 5' intron, a chimeric IgG_1 antibody heavy chain, and a portion of a genomic sequence from the region just downstream of the human IgG_4 constant region containing a polyA signal. Replacement of the heavy chain variable region with that from 1F6 resulted in a plasmid carrying the chimeric 1F6 sequence. To prepare this construct, the entire1F6 heavy chain variable region sequence was amplified via PCR from the sequencing vector. The

forward oligonucleotide primer 5'-ATA AAT AAG CTT ACC GCC ACC ATG GCT TGG GTG TGG ACC TTG-3' (SEQ ID NO:46) encoding a HindIII restriction site, a consensus Kozac sequence 5' to the coding sequence for the heavy chain leader, and sequence homologous to the leader sequence was used. The reverse primer 5'-ATA AAG GCT AGC TGA GGA GAC GGT GAC TGA GGT-3' (SEQ ID NO:47) encoded sequence homologous to the 3' end of the variable region and a NheI restriction site. The PCR product was digested with HindIII and NheI. The pSG850 vector was digested with the same enzymes to remove the existing variable region and the larger vector fragment was isolated. Ligation of the 1F6 VH and the pSG850 vector fragments resulted in the construction of pJC140 containing the chimeric 1F6 heavy chain.

[0195] *Construction of the light chain chimerization vector*

[0196] A chimerization vector, pSG855, had been previously constructed by cloning a 4 kb XhoI-XbaI fragment of a CHEF1 vector into a Bluescript vector. This fragment contains a portion of the human IgG_4 downstream region, the CHEF1 promoter region, the CHEF1 5'intron, a chimeric antibody kappa light chain, and the human kappa downstream region containing a polyA signal. Replacement of the existing light chain variable region with that from 1F6 resulted in a plasmid carrying the chimeric 1F6 kappa sequence. To prepare this construct, the entire 1F6 light chain variable region sequence was amplified via PCR from the sequencing vector. The forward oligonucleotide primer 5'-ATA AAG AAG CTT ACC GCC ACC ATG GAG ACA GAC ACA CTC CTG-3' (SEQ ID NO:48) encoding a HindIII restriction site, a consensus Kozac sequence 5' to the coding sequence for the light chain leader, and sequence homologous to the leader sequence was used. The reverse primer 5'-ATA AAG GAA GAC AGA TGG TGC AGC CAC AGT CCG TTT GAT TTC CAG CTT GGT GCC-3' (SEQ ID NO:49) encoded sequence complementary to the last 24 base pairs of the light chain variable region and the first 24 base pairs of the kappa constant region, including a BbsI restriction site. The PCR product was digested with HindIII and BbsI. The pSG855 vector was digested with the same enzymes to cut out the existing variable region and the larger vector fragment was isolated. Ligation of the 1F6 VL and the pSG855 vector fragments resulted in the construction of pJC160 containing the chimeric 1F6 light chain.

[0197] *Assembly of the c1F6 expression vector*

[0198] The expression vector carrying both chains of the chimeric 1F6 antibody was assembled via a three-way ligation. The CHEF1 expression vector pDEF14 was digested with NotI and XbaI, and the 19.7 kb vector fragment was isolated. pJC140 was digested with NotI and XhoI and the 6 kilobase fragment was isolated. pJC160 was digested with XhoI and XbaI and the 4 kilobase fragment was isolated. These three fragments were mixed in a 1:1:1 molar ratio in a ligation reaction, and the ligation product was used to transform XL10-Gold cells. Clones were screened by restriction mapping, and a correct clone was confirmed by sequencing of the heavy and light chain coding regions. Figure 4 shows the plasmid map of the final product.

[0199] *Transfection of DG44 cells with pDEF14-1F6*

[0200] 200 μg of pDEF14-1F6 plasmid DNA was linearized with PvuI overnight at 37°C, and then ethanol precipitated after the addition of 100 μg sonicated salmon sperm DNA (Specialty Media cat# S-005-G Lavallette, NJ). The DNA was resuspended in 350 μl sterile dH_2O and 450 μl 2X HeBS (40 mM HEPES-NaOH pH 7.0, 274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, and 12 mM dextrose).

[0201] DG44 Chinese Hamster Ovary cells were from a bank of cells which had been previously adapted to serum-free suspension. The DG44 were cultured in shake flasks to a density of approximately 1 x 10^6 cells/ml in nonselective media, serum-free Excell325 (JRH Biosciences, Inc., Lenexa, KS) supplemented with recombinant human insulin, L-glutamine, hypoxanthine and thymidine. 2 x 10^7 cells were harvested by centrifugation in sterile 15 ml tubes, washed with CMF-PBS and repelleted. The washed DG44 cells were resuspended in the DNA solution and pulsed once in a BioRad GenePulser II electroporator (960 μF, 290 volts, time constant 9 - 11 msec) (Bio-Rad, Hercules, CA). The cells were allowed to recover at room temperature for 8 - 10 minutes. Cells were then added to 10 ml of the non-selective, serum-free medium above, transferred to stationary T75 flasks and allowed to recover for two days at 37°C and 5% CO_2. The transfection pool was then centrifuged at low speed and resuspended in selective medium (same as the medium described above except without hypoxanthine and thymidine, designated Excell325SEL) and passaged until the culture reached a viability of >90%. A fed-batch culture of this transfection pool yielded cAb with specificity comparable to the murine 1F6 Ab.

[0202] *Subcloning of c1F6 transfection pool*

[0203] Using a feeder-cell method, the c1F6 transfection pool cells were mixed with DG44 cells at a ratio of 1:2000 in Excell325SEL (without hypoxanthine or thymidine) and plated at a density of 1000 cells/well into four 96-well plates. This gave an effective density of 0.5 c1F6 cells/well along with 1000 DG44 feeder cells/well. The DG44 cells died off after a few days due to their requirement for HT. DG44 cells plated at 10,000 cells/well under the same conditions were shown to exhibit no survival. The two plates yielded 34 wells with outgrowth of single colonies. The 34 clonal wells were scaled up to 24–well plates and extinct-culture supernatants from the wells were screened for cAb titer via a standard anti-chimeric antibody ELISA. Six subclones were chosen based on titer and growth for further screening via shake flask fed-batch culture. One of these clones demonstrated a cell-specific productivity of approximately 20 picograms per cell per day, with a final fed-batch titer of 1.1 g/l.

[0204] *Example 2: Chimeric 1F6 mediates ADCC against $CD70^+$ tumor cell lines.*

[0205] The ability of c1F6 to mediate ADCC against the $CD70^+$ cell lines WIL2-S, Caki-1 and 786-O was measured using a standard ^{51}Cr release assay. Tumor cells were labeled for one hour with 100 μCi $Na_2{}^{51}CrO_4$, washed thoroughly to remove unincorporated radioisotope, then plated in a 96-well plate at a concentration of 5,000 cells per well. Antibodies c1F6, m1F6 or human Ig were added to appropriate wells at a final concentration of 1 μg/ml for 0.5 hours prior to the addition of effector PBMC. PBMC were adjusted to reflect an effector cell to target cell ratio of 30 $CD16^+$ cells:1 target cell. After 4 hours incubation, the ^{51}Cr released from lysed cells was measured and the percent specific lysis calculated as {(test sample cpm – spontaneous cpm) ÷ (total cpm – spontaneous cpm)} X 100. Spontaneous release of isotope was determined from the supernatant of target cells incubated in medium alone. Total counts were determined from target cells lysed with 2% triton-X. As shown in Figure 5, c1F6 effectively induced the lysis of each tumor target whereas tumor cells treated with CD70-binding murine 1F6 (m1F6) or non-binding control human Ig (hIg) were minimally affected. Lysis of the NK sensitive target K562, in the absence of antibody, confirmed the presence of effector cells with cytolytic potential within PBMC.

[0206] *Example 3: Chimeric 1F6-coated target cells recognized by PBMC from multiple donors.*

[0207] Caki-1 renal cell carcinoma cells were labeled with $Na_2{}^{51}CrO_4$, treated with graded doses of c1F6, and then incubated with effector PBMC from two normal donors. Specific lysis was assessed after 4 hours incubation as described in Example 2. Donor 2051661 and ND016 efficiently lysed Caki-1 target cells treated with 1 or 0.1 μg/ml c1F6 (Figure 6). Specific lysis decreased in an antibody dose-dependent manner thereafter, and was negligible when target cells were treated with 0.001 μg/ml c1F6. Target cells mixed with non-binding control Ig (hIg) were not lysed by PBMC from either donor.

[0208] *Example 4: Chimeric 1F6-coated lymphoid cell lines are susceptible to lysis by PBMC.*

[0209] To determine if transformed cells of B and T cell lineage were also susceptible to c1F6-mediated ADCC activity, $CD70^+$ B lymphoblastoid cells (WIL2-S) and cutaneous T cell lymphoma cells (HH) were labeled with $Na_2{}^{51}CrO_4$ and then mixed with c1F6 or human Ig at various concentrations, as indicated. PBMC were added to the target cells at a ratio of 18:1 ($CD16^+$ cells:target) and percent lysis determined after a four-hour incubation as described in Example 2. Figure 7 shows that both WIL2-S and HH are recognized and lysed by PBMC effector cells in the presence of c1F6. Whereas treatment of target cells with non-binding control antibody resulted in minimal cell lysis, treatment of WIL2-S and HH cells with c1F6 at 1 μg/ml enabled 43.5% and 37.5% target cell death, respectively.

[0210] The multiple myeloma cell lines L-363, JJN-3, LP-1 and U-266 were tested for expression of CD70 by flow cytometry. As shown in Figure 8A, CD70 was readily detected on each. The susceptibility of these multiple myeloma cell lines to chimeric 1F6-mediated ADCC was determined as described in Example 2. Target cells treated with 1 or 0.1 μg/ml c1F6 were efficiently lysed (Figure 8B). Specific lysis decreased in an antibody dose-dependent manner thereafter, and was negligible when target cells were treated with 0.001 μg/ml c1F6. Target cells mixed with non-binding control Ig (hIg) were not lysed. Blockade of FcγRIII by pre-incubating effector cells with anti-CD16 antibody abolished ADCC activity, confirming the dependence of lytic activity on interaction of antibody with FcR-

bearing effector cells. Similar dose-dependent chimeric 1F6-mediated ADCC was also observed using $CD70^+$ Hodgkin's disease cell lines as targets (Figure 9).

[0211] *Example 5: CD70 Expression on Activated T Cells During an Antigen-Specific In Vitro Immune Response.*

[0212] A 9-amino acid peptide (GILGFVFTL, M1 peptide, (SEQ ID NO:50)) derived from the influenza virus matrix protein binds to the peptide-binding groove of the HLA-A0201 molecule. Presentation of the M1 peptide by HLA-A0201 expressing antigen presenting cells to autologous T cells specifically stimulates the activation and expansion of $CD8^+$ cytotoxic T cells expressing the T cell receptor Vβ17 chain (Lehner *et al.*, 1995, *J. Exp. Med.* 181: 79-91), constituting a convenient *in vitro* experimental system to track the activation and expansion of antigen-specific T cells to their cognate antigen.

[0213] To examine CD70 expression on activated antigen-specific T cells, PBMCs from a normal donor expressing HLA-A0201 were stimulated with the M1 peptide. PBMCs were seeded at 2×10^6 cells/ml with 5 μg/ml of M1 peptide in AIMV medium supplemented with 5% human AB serum. IL-2 (Proleukin, Chiron, Emeryville, CA) and IL-15 (R and D Systems, Minneapolis, MN) were added to a final concentration of 20 IU/ml and 5 ng/ml, respectively, once every two days beginning on day 2 after culture initiation. The expansion of $CD8^+/V\beta17^+$ T cells and induction of CD70 on the $CD8^+/V\beta17^+$ were followed by three-color flow cytometry. $V\beta17^+$ T cells were identified by the anti-TCRVβ17 mAb clone E17.5F3 (Beckman Coulter, Miami, FL). Results showed that only 0.9% of the cells within the lymphocyte population was $CD8^+/V\beta17^+$ two days after culture initiation. T cell expansion was only evident within the $CD8^+/V\beta17^+$ population. The percentage of $CD8^+/V\beta17^+$ progressively increased to 1.9% on day 5, to 14% on day 7, and to 23% on day 11. CD70 expression became detectable 3 days after antigen stimulation and increased to approximately 60% of the expanding $CD8^+/V\beta17^+$ cells on day 7 (Figure 10A). The highest level of CD70 expression as indicated by the mean fluorescence intensity (MFI) was also detected on day 7 (Figure 10B). The percentage of $CD70^+/CD8^+/V\beta17^+$ cells and the MFI of CD70 expression on the $CD8^+/V\beta17^+$ started to decline thereafter. Whereas CD70 was clearly expressed on the $CD8^+/V\beta17^+$ cells, no CD70 could be detected on the $CD8^+/V\beta17^-$

cells. These results confirmed that CD70 was induced on activated T cells responding to the antigenic stimulation but not the bystander, antigen non-specific T cells.

[0214] *Example 6: In Vitro Deletion of CD70$^+$ Antigen-Specific T Cells by c1F6.*

[0215] To test the ability of c1F6 to deplete antigen-specific activated T cells, PBMC from a normal donor expressing HLA-A0201 were stimulated with the M1 peptide in the presence or absence of anti-CD70 antibody. PBMC were seeded in a 24-well plate at a concentration of 0.5 x 10^6 cells/ml with 5 μg/ml M1 peptide in 2 ml medium supplemented with IL-2 and IL-15, as described *supra*. Nonbinding immunoglobulin, murine 1F6 (m1F6) or chimeric 1F6 (c1F6) antibody was added at a concentration of 1 μg/ml at the start of the culture and on days 2 and 5 post culture initiation. On day 5, half of the spent culture supernatant was replaced with fresh cytokine-containing medium. On day 9, the percentage of antigen-reactive cells (CD8$^+$/Vβ17$^+$ population) was determined by flow cytometric analysis of cells stained with FITC-conjugated anti-Vβ17- and PE-Cy5-conjugated anti-CD8 antibodies. Some of the cell cultures were additionally supplemented on days 0 and 5 with 0.25 x 10^6 CD16$^+$ cell-enriched PBMC. To enrich for CD16$^+$ cells, T cells, B cells and monocytes were depleted by labeling PBMC with anti-CD8, anti-CD4, anti-CD20, and anti-CD14 antibodies followed by immunomagnetic bead selection. Over the course of the study, the antigen-specific CD8$^+$/Vβ17$^+$ cells expanded to comprise 47.2% of all viable cells within the culture in the absence of antibody. Similarly, CD8$^+$/Vβ17$^+$ cells represented 48.3% and 38.5% of all cells present in cultures treated with the nonbinding antibody and murine anti-CD70 antibody, respectively. In contrast, substantial inhibition of CD8$^+$/Vβ17$^+$ expansion was observed in cultures treated with chimeric anti-CD70 antibody (12.5%). The reduction in antigen-reactive cells was even more pronounced when CD16$^+$ effector cells were added to the culture. CD8$^+$/Vβ17$^+$ cells made up only 1.5% of all cells in c1F6-treated cultures compared to 32.6% in untreated cultures and 22.4% or 22.9% of cells treated with irrelevant and m1F6 antibodies, respectively. These results demonstrate that c1F6 selectively targets and prevents the expansion of antigen-activated T cells.

[0216] *Example 7: Dose response comparison of anti-CD70 on depletion of antigen-specific CD8+/Vβ17+ cells.*

[0217] To confirm the ability of c1F6 to prevent the expansion of antigen-activated T cells and to further evaluate the antibody-dependent nature of this response, a second study was performed in which M1-peptide stimulated PBMCs were initiated in the presence of graded doses of c1F6 (Figure 11). Antigen-specific $CD8^+/V\beta17^+$ cells recovered on day 9 from untreated cultures represented 56% of all viable cells. In contrast, addition of c1F6 to the cultures on day 0 significantly limited expansion of the antigen-reactive population in a dose dependent manner. $CD8^+/V\beta17^+$ cells comprised 7.8, 5.8, and 16.9% of all cells in cultures treated with 1, 0.1 and 0.01 μg/ml c1F6, respectively. c1F6 antibody added at a concentration of 0.001 μg/ml did not prevent $CD8^+/V\beta17^+$ cell expansion.

[0218] *Example 8. Chimeric 1F6 mediates complement-dependent cytotoxicity in $CD70^+$ B and T cells.*

[0219] The ability of c1F6 to mediate complement-dependent cytotoxicity was examined using $CD70^+$ B and T cells. In these experiments, normal human serum that was not heat-inactivated was used as the source of complement. Target cells were treated with graded doses of c1F6 or a non-binding human IgG control in the presence of normal human serum. After incubation at 37°C for 2 hours, propidium iodide was added to a final concentration of 5 μg/ml. Cell preparations were then examined by flow cytometry. Cells stained by propidium iodide were considered to have lost plasma membrane integrity (cell lysis) as a result of antibody-mediated complement activation and formation of the membrane attach complex. Spontaneous background lysis was subtracted from antibody-mediated cell lysis to yield specific cell lysis. Using this assay, c1F6 mediated dose-dependent lysis of several $CD70^+$ B cell targets (Figure 12). These targets included the lymphoblastic non-Hodgkin's lymphoma line (MHH-PREB-1), the EBV- Burkitt's lymphoma line (MC116), and the EBV^+ lymphoblastoid B cell line (WIL2-S). For both MHH-PREB-1 and WIL2-S, maximum specific lysis was >50%. Specific lysis of MC116 was around 15% at the highest concentration of c1F6 used.

[0220] The sensitivity of two $CD70^+$ T cell targets was also examined. HH is a cutaneous T cell lymphoma cell line that constitutively expresses CD70. C9D is a normal T cell line that is maintained and propagated in culture. Stimulation of resting C9D with phytohemagglutinin (PHA, 2 μg/ml), IL-2 (100 IU/ml Proleukin® (aldesleukin) Chiron, Emeryville, CA), and irradiated CESS cells (ATCC, Manassas, VA) at a 1:1 CESS:T cell

ratio initiates a cell activation and expansion cycle which is accompanied by inducible surface expression of CD70. HH and $CD70^+$ C9D cells were incubated with c1F6 or control IgG as described above in the presence of normal human serum. Cell lysis was assessed by propidium iodide permeability and flow cytometry (Figure 13). Cell lysis was detected in both targets in a dose-dependent manner, demonstrating that c1F6 also mediates CDC on $CD70^+$ T cell targets.

[0221] *Example 9: Chimeric 1F6 mediates ADCP against CD70$^+$ tumor cell lines.*

[0222] The ability of c1F6 to mediate antibody-dependent cellular phagocytosis was examined using CD70$^+$ WIL2-S cells and monocyte-derived macrophages as a source of phagocytic cells. To generate macrophages, monocytes from PBMC were adhered to tissue culture flasks for approximately 1 hour in medium containing 1% human serum. Nonadherent cells were decanted and the remaining adherent cells cultured in serum-free X-VIVO medium (BioWhittaker, Walkersville, MD) supplemented with 500 units/mL rhGM-CSF for 11-14 days. Macrophages were ≥ 75% viable, were CD3$^-$/CD14$^+$/CD11b$^+$, and expressed FcγRI, II and III. To detect c1F6-mediated ADCP, 8x10^5 WIL2-S target cells were stained with PKH67 (Sigma-Aldrich Corp., St. Louis, MO), a green fluorescent cell membrane dye, according to the manufacturer's protocol. The cells were then pre-incubated with 2ug/mL of c1F6 in PBS for 30 minutes on ice and washed once with PBS to remove excess antibody. The target cells were combined with 2x10^5 macrophages in a 96-well U-bottom microtiter plate at a final ratio of 1 macrophage cell to 4 target cells in RPMI 1640 medium supplemented with 10% Ultra Low IgG FBS (Invitrogen Corp.). After a two-hour incubation at 37°C in a 5% CO_2 humidified incubator, the cell mixture was labeled with PE-conjugated mouse anti-CD11b antibody to surface label the macrophages. The cells were washed once with PBS, fixed in 1% paraformaldehyde in PBS, and analyzed by flow cytometry to detect double fluorescence as a measure of phagocytic activity. For fluorescence microscopy, CD11b$^+$ cells were further labeled with Alexa Fluor™ 568 goat anti-mouse IgG (Molecular Probes, Inc., Eugene, OR) to enhance the red fluorescent signal. As shown in Figure 14A, 79% of macrophages phagocytosed WIL2-S target cells when the targets were coated with c1F6. In contrast, limited phagocytosis was observed by macrophages mixed with WIL2-S cells treated with non-binding Ig control antibody (12.8%). The double fluorescence staining indicative of phagocytosis was a result of target cell ingestion and was not due to conjugate formation, as judged by fluorescent microscopy. Green WIL2-S cellular material was clearly shown to be localized within macrophages whose membranes were stained red. WIL2-S cells treated with non-binding Ig were seen to be separate and apart from red-stained macrophages. Phagocytic activity was dependent upon antibody in a dose specific manner (Figure 14B). Further examination revealed that CD70$^+$ transformed cell lines derived from different cancer types were all sensitive to chimeric 1F6-mediated ADCP (Figure 15).

[0223] *Example 10: In vivo antitumor activity of c1F6.*

[0224] CD70 positive Burkitt's lymphoma line Raji and EBV-transformed lymphoblastoid cell line IM-9 were obtained from the ATCC (Manassas, VA). Cells were grown in RPMI (Life Technologies Inc., Gaithersburg, MD) and supplemented with 10% Fetal Bovine Serum. To establish disseminated disease, 1 x 10^6 Raji or IM-9 cells washed and resuspended in 0.2 ml PBS were injected into the lateral tail vein of C.B.-17 SCID mice (Harlan, Indianapolis, IN). After injection, all of the mice were pooled and then placed randomly into the various treatment groups. A single dose of 1 or 4 mg/kg of c1F6, or 4 mg/kg of control IgG was given one day after the cell implant by intravenous injection into the lateral tail vein. Mice were weighed and evaluated for signs of disease at least once per week. Mice were removed from the experiment and sacrificed when they exhibited signs indicative of disease onset characterized by one or more of the following: weight loss of 15-20% from day 0 body weight, hunched posture, lethargy, cranial swelling, or dehydration. In the Raji model median survival for the untreated and control IgG-treated groups was 21 and 24 days, respectively. Chimeric 1F6 prolonged survival in a dose-dependent manner, with a median survival of 31 and 72 days in the groups treated at 1 and 4 mg/kg of chimeric 1F6, respectively (Figure 16). A similar increase in survival was also observed in the IM-9 model. In the absence of treatment or treatment with control IgG, the median survival was 35 and 28 days, respectively. Treatment with 1 or 4 mg/kg of c1F6 increased the median survival time to 53 and > 100 days, respectively (Figure 16). In both models the increase in survival was found to be statistically significant based on the log-rank test as indicated in Figure 16.

[0225] *Example 11: Treatment of Experimental Allergic Encephalomyelitis by Administration of Anti-CD70 Antibodies*

[0226] Studies indicate a role for CD70/CD27-mediated T cell-T cell interactions in enhancing the Th_1-mediated immune responses in cell-mediated autoimmune diseases, including, for example, autoimmune demyelinating diseases. In this example, experimental allergic encephalomyelitis (EAE), an animal model of the demylelinating disease multiple sclerosis (MS), is treated with a chimeric or humanized anti-CD70 antibody that recognizes an epitope of murine CD70 correspoding the 1F6 epitope of human CD70.

[0227] *Induction and clinical assessment of Experimental Allergic Encephalomyelitis (EAE)*: R-EAE (relapsing EAE) is induced in six- to seven-week-old female SJL mice by subcutaneous immunization with 100 μl of complete Freund's adjuvant (CFA) emulsion containing 200 μg of *Mycobacterium tuberculosis* H37Ra and 40 μg of the immunodominant epitope of proteolipid protein, $PLP_{139-151}$. The signs of EAE are scored on a 0 to 5 scale as follows: (0) normal; (1) limp tail or hind limb weakness; (2) limp tail and hind-limb weakness (waddling gait); (3) partial hind-limb paralysis; (4) complete hind-limb paralysis; and (5) moribund. A relapse is defined as a sustained increase (more than 2 days) in at least one full grade in clinical score after the animal had improved previously at least a full clinical score and had stabilized for at least 2 days. The data are plotted as the mean clinical score for all animals in a particular treatment group or as the relapse rate (total number of relapses in a group divided by the total number of mice in that group).

[0228] *Anti-CD70 Administration Regimens*: Anti-CD70 antibody (0.1-3 mg/kg body weight) is administered intraperitoneally in a total volume of 100 μl. Mice are treated 3 times per week for 3 consecutive weeks (9 total treatments). Treatment is initiated before disease onset (day 7) or at the peak of acute disease (day 14). As a control, one group of EAE-induced mice are left untreated.

[0229] *Inhibition of TNF-α and IFN-γ induction*: Demonstration of the presence of TNF-α and IFN-γ in the brains of mice with EAE shows an inflammatory disease process indicative of EAE disease progression. Inhibition of the induction of these cytokines in the brains of SJL mice treated with anti-CD70 antibody indicates the value of anti-CD70 antibody therapy in preventing or treating EAE. Brains are obtained from from at least three animals treated preclinically (at day 13, after three treatments, and day 26, after nine treatments) and at peak of acute disease (at day 20, after three treatments, and day 33, after nine treatments). Brains are fixed (10% buffered formalin), and tissues are embedded in paraffin and sectioned. Sections are then independently stained for TNF-α or IFN-γ by incubation with a primary antibody specific for the respective cytokine, followed by incubation with a secondary antibody conjugated to FITC. Tissue sections are then mounted in mounting media and analyzed by immunofluorescence microscopy. Decreased TNF-α or INF-γ staining in anti-CD70 antibody-treated mice versus the untreated EAE-induced mice shows inhibition of inflammatory cytokine induction using anti-CD70 antibody therapy.

[0230] *Inhibition of Disease Symptoms or Relapse Rates*: EAE-induced SJL mice in the anti-CD70 antibody treatment group are compared with untreated EAE-induced mice to assess the efficacy of anti-CD70 antibody therapy in either preventing disease onset or treating established disease. For mice treated preclinically, a decrease in the mean score for EAE disease, as compared to the untreated control group, demonstrates the efficacy of anti-CD70 antibody therapy in preventing disease. For mice treated at the peak of acute disease, either (a) a decrease in the relapse rate or (b) a decrease in the post-treatment mean score for EAE, as compared to the untreated control group, demonstrates the efficacy of anti-CD70 antibody therapy in treating established disease.

[0231] *Example 12: Treatment of Graft versus Host Disease by Administration of Anti-CD70 Antibodies*

[0232] The hu-SCID model has proven to be an effective system for investigating human immunological diseases. In this model of graft versus host disease, the effects of anti-CD70 antibodies and antibody drug conjugates on human PBLs and/or PBMCs are studied.

[0233] Establishment of Human Immune Cells in Immunodeficient Mice: Prior to injection of human PBL or PBMC, the following effector cells are depleted in the mice with the indicated reagents: for Natural Killer (NK) cells, *e.g.*, anti-asialo-GM1 or TMB-1 antibody; for macrophages/monocytes, *e.g.*, chlodronate encapsulated liposomes; for neutrophils, *e.g.*, anti-Gr-1 antibody; for complement, *e.g.*, cobra venom factor. Human PBL or PBMC (1-30x10^7) are transplanted into SCID mice (female CB.17-SCID, SCID-NOD, or CB.17-SCID/beige mice, 8-12 weeks old) to establish a stable long-term reconstitution of a functional human immune system in the mice.

[0234] Human cell engraftment in SCID mice is assessed by analyzing sera for the presence human immunoglobulin during the experiment. Engraftment efficiency is also measured by human cell counts in blood, peritoneal exudates, and spleens of anesthetized or euthanized animals throughout the study. Upon successful establishment of human cell engraftment, the mice are treated with anti-CD70 antibody or anti-CD70 antibody drug conjugate (1-10 mg/kg body weight, given intraperitoneally or intravenously, four-seven doses every four to seven days). The effects of antibody or antibody conjugate treatment on human cells is investigated by examining the numbers of the human cells in mouse blood and/or spleen taken at different days (1, 4, 7, 14, and 28) after treatment.

[0235] *Collection of tissues:* At specific time points post injection and at the end of the experiment, the following tissues are collected and analyzed for disease progression and cellular infiltration from euthanasized mice: spleen, lymph nodes, thymus, liver, bone marrow, lung, brain, intestine, colon, skin, pancreas, peritoneal exudate, and blood.

[0236] *Example 13: Treatment of Astma by Administration of Anti-CD70 Antibodies.*

[0237] The efficacy of anti-CD70 antibody was investigated in a murine model of asthma. Balb/c mice were treated with 10 mg OVA/Alum for sensitization at days 0, 7 and 14. An anti-murine CD70 antibody (clone 3B9) at a dose of 10 mg/kg body weight, was administered intraperitoneally Q7dx4 starting at day 0. The mice were then challenged with 5% aerosolized ovalbumin at days 21, 22, and 23. On day 26, the mice were sacrificed and the bronchioalveolar lavage fluid, blood, draining lymph nodes, spleen, and lung were collected. The results obtained indicated a milder cellular infiltration to the lung in the 3B9 treated group in comparison to the control group.

[0238] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME	1	DE	2
CONTENANT LES PAGES	1	À	72

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME	1	OF	2
CONTAINING PAGES	1	TO	72

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. Use of a humanized or chimeric antibody for the treatment of a CD70-expressing cancer in a subject, wherein the antibody comprises

an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first complementarity determining region (CDR) having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

2. Use of a humanized or chimeric antibody in the manufacture of a medicament for the treatment of a CD70-expressing cancer in a subject, wherein the antibody comprises an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP, or CDC response in the subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

3. The use of claim 1 or claim 2 , wherein the antibody is a chimeric antibody.

4. The use of claim 1 or claim 2, wherein the antibody is a humanized antibody.

5. The use of claim 3 or claim 4, wherein the antibody comprises an effector domain of a human IgG antibody.

6. The use of claim 5, wherein the IgG antibody is of the human IgG1 or IgG3 subtype.

7. The use of claim 1 or claim 2 , wherein the antibody comprises a heavy chain variable region having the amino acid sequence set forth in residues 20-137 of SEQ ID NO:2.

8. The use of claim 7, wherein the antibody further comprises a light chain variable region having the amino acid sequence set forth in residues 21-132 of SEQ ID NO:12.

9. The use of claim 1 or claim 2, wherein the antibody is multivalent.

10. The use of claim 1 or claim 2, wherein the CD70-expressing cancer is selected from the group consisting of a multiple myeloma, kidney tumor, a B cell lymphoma, a colon carcinoma, Hodgkin's Disease, non-Hodgkins lymphoma, a nasopharyngeal carcinoma, brain tumor and a thymic carcinoma.

11. The use of claim 10, wherein the kidney tumor is a renal cell carcinoma.

12. The use of claim 10, wherein the brain tumor is a glioma, a glioblastoma, or a meningioma.

13. The use of claim 1 or claim 2, wherein the subject is human.

14. Use of a humanized or chimeric antibody for the treatment of an immunological disorder characterized by expression of CD70 on immune cells in a subject, wherein the antibody comprises:

an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in the subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

15. Use of a humanized or chimeric antibody in the manufacture of a medicament for the treatment of an immunological disorder characterized by expression of CD70 on immune cells in a subject, wherein the antibody comprises an antigen-binding region that binds to CD70 and at least one effector domain mediating at least an ADCC, ADCP, or CDC response in the subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect and is not conjugated to a therapeutic agent.

16. The use of claim 14 or claim 15, wherein the antibody is a chimeric antibody.

17. The use of claim 14 or claim 15, wherein the antibody is a humanized antibody.

18. The use of claim 16 or claim 17, wherein the antibody comprises an effector domain of a human IgG antibody.

19. The use of claim 18, wherein the IgG antibody is of the human IgG1 or IgG3 subtype.

20. The use of claim 14 or claim 15, wherein the antibody comprises a heavy chain variable region having the amino acid sequence set forth in residues 20-137 of SEQ ID NO:2.

21. The use of claim 20, wherein the antibody further comprises a light chain variable region having the amino acid sequence set forth in residues 21-132 of SEQ ID NO:12.

22. The use of claim 14 or claim 15, wherein the antibody is multivalent.

23. The use of claim 14 or claim 15, wherein the immunological disorder is a T cell-mediated immunological disorder.

24. The use of claim 23, wherein the T cell mediated immunological disorder comprises activated T cells expressing CD70.

25. The use of claim 24, wherein resting T cells are not substantially depleted by administration of the antibody.

26. The use of claim 23, wherein the T cell-mediated immunological disorder is rheumatoid arthritis, systemic lupus erythematosus (SLE), Type I diabetes, asthma, atopic dermatitis, allergic rhinitis, immune thrombocytopenic purpura, multiple sclerosis, psoriasis, Sjorgren's syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis,

Wegener's granulomatosis, tuberculosis, Waldenstrom's macroglobulemia, or graft versus host disease.

27. The use of claim 14 or claim 15, wherein the immunological disorder is an activated B-lymphocyte disorder.

28. The use of claim 14 or claim 15, wherein the subject is human.

29. A humanized or chimeric antibody comprising an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect on a CD70 expressing cancer, which cytotoxic effect is achieved in the absence of conjugation to a cytotoxic agent.

30. The antibody of claim 29, wherein the antibody is a humanized antibody.

31. The antibody of claim 30, wherein the antibody is of the human IgG1 or IgG3 subtype.

32. A humanized or chimeric antibody comprising an antigen-binding region that binds to CD70, and at least one effector domain mediating at least an ADCC, ADCP or CDC response in a subject,

wherein the antigen-binding region comprises a heavy chain variable region comprising a first CDR having the amino acid sequence of residues 45-54 of SEQ ID NO:2, a second CDR having the amino acid sequence of SEQ ID NO:8, and a third CDR having the

amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a first CDR having the amino acid sequence of SEQ ID NO:16, a second CDR having the amino acid sequence of SEQ ID NO:18, and a third CDR having the amino acid sequence of SEQ ID NO:20, and

wherein the effector domain is the effector domain of a human IgM or IgG antibody, and the antibody exerts a cytotoxic effect on a CD70 expressing immune cell, which cytotoxic effect is achieved in the absence of conjugation to a cytotoxic agent.

33. The antibody of claim 32, wherein the antibody is a humanized antibody.

34. The antibody of claim 33, wherein the antibody is of the human IgG1 or IgG3 subtype.

35. A pharmaceutical composition for the treatment of a CD70-expressing cancer or an immunological disorder characterized by CD70-expressing immune cells, the composition containing an antibody of any one of claims 29 to 34, and at least one pharmaceutically compatible ingredient.

36. A pharmaceutical kit, comprising:

a container comprising the antibody of any one of claims 29 to 31 or claims 32 to 34, wherein the antibody is lyophilized, and

a second container comprising a pharmaceutically acceptable diluent.

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Figure 1

1F6 VL nucleotide sequence:

```
1   atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt  60
61  gacattgtgc tgacacagtc tcctgcttcc ttagctgtat ctctggggca gagggccacc 120
121 atctcatgca gggccagcaa aagtgtcagt acatctggct atagttttat gcactggtat 180
181 caacagaaac caggacagcc acccaaactc ctcatctatc ttgcatccaa cctagaatct 240
241 ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caacatccat 300
301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga ggttccgtgg 360
361 acgttcggtg gaggcaccaa gctggaaatc aaacgg                           396
```

1F6 VL amino acid sequence:

```
-20 METDTLLLWV LLLWVPGSTG                                            -1
1   DIVLTQSPAS LAVSLGQRAT ISCRASKSVS TSGYSFMHWY QQKPGQPPKL LIYLASNLES  60
61  GVPARFSGSG SGTDFTLNIH PVEEEDAATY YCQHSREVPW TFGGGTKLEI KR         112
```

1F6 VH nucleotide sequence:

```
1   atggcttggg tgtggacctt gctattcctg atggcagctg cccaaagtgc ccaagcacag  60
61  atccagttgg tgcagtctgg acctgaggtg aagaagcctg gagagacagt caagatctcc 120
121 tgcaaggctt ctgggtatac cttcacaaac tatggaatga actgggtgaa gcaggctcca 180
181 ggaaagggtt taaagtggat gggctggata aacacctaca ctggagagcc aacatatgct 240
241 gatgccttca agggacggtt tgccttctct ttggaaacct ctgccagcac tgcctatttg 300
301 cagatcaaca acctcaaaaa tgaggacacg gctacatatt tctgtgcaag agactacggc 360
361 gactatggta tggactactg gggtcaagga acctcagtca ccgtctcctc a         411
```

1F6 VH amino acid sequence:

```
-19 MAWVWTLLFL MAAAQSAGA                                             -1
1   QIQLVQSGPE VKKPGETVKI SCKASGYTFT NYGMNWVKQA PGKGLKWMGW INTYTGEPTY  60
61  ADAFKGRFAF SLETSASTAY LQINNLKNED TATYFCARDY GDYGMDYWGQ GTSVTVSS   118
```

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Figure 2

2F2 VL nucleotide sequence:

```
1   atggagacag acacactcct gttatgggta ctgctgctct gggttccagg ttccactggt  60
61  gacattgtgc tgacacagtc tcctgcttcc ttaactgtat ctctggggca gaagaccacc 120
121 atctcatgca gggccagcaa gagtgtcagt acatctggct atagttttat gcactggtac 180
181 caactgaaac caggacagtc acccaaactc ctcatctatc ttgcgtccaa cctaccatct 240
241 ggggtccctg ccaggttcag tggcagtggg tctgggacag acttcaccct caaaatccat 300
301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc acagtaggga gattccgtac 360
361 acgttcggag gggggaccaa gctggaaata acacgg                           396
```

2F2 VL amino acid sequence:

```
-20 METDTLLLWV LLLWVPGSTG                                            -1
1   DIVLTQSPAS LTVSLGQKTT ISCRASKSVS TSGYSFMHWY QLKPGQSPKL LIYLASNLPS  60
61  GVPARFSGSG SGTDFTLKIH PVEEEDAATY YCQHSREIPY TFGGGTKLEI TR         112
```

2F2 VH nucleotide sequence:

```
1   atggaatgga cctgggtctt tctcttcctc ctgtcagtaa ctgcagatgt ccaatcccag  60
61  gttcagctgc aacagtctgg aactgagctg atgacgcctg gggcctcagt gacgatgtcc 120
121 tgcaagactt ctggctacac attcagtacc tactggatag agtgggtaaa acagaggcct 180
181 ggacatggcc ttgagtggat tggagaaatt ttacctggaa gtggttatac tgactacaat 240
241 gagaagttca aggccaaggc cacattcact gcagatacat cctccaacac agcctacatg 300
301 caactcagca gcctggcatc tgaggactct gccgtctatt actgtgcaag atgggatagg 360
361 ctctatgcta tggactactg gggtcaagga acctcagtca ccgtctcctc a          411
```

2F2 VH amino acid sequence:

```
-19 MEWTWVFLFL LSVTADVQS                                             -1
1   QVQLQQSGTE LMTPGASVTM SCKTSGYTFS TYWIEWVKQR PGHGLEWIGE ILGPSGYTDY  60
61  NEKFKAKATF TADTSSNTAY MQLSSLASED SAVYYCARWD RLYAMDYWGG GTSVTVSS   118
```

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Figure 3

1F6 CDR-L1 (residues 23 to 38): R A S K S V S T S G Y S F M H
2F2 CDR-L1 (residues 23 to 38): R A S K S V S T S G Y S F M H

1F6 CDR-L2 (residues 54 to 60): L A S N L *E* S
2F2 CDR-L2 (residues 54 to 60): L A S N L *P* S

1F6 CDR-L3 (residues 93 to 101): Q H S R E V P W T
2F2 CDR-L3 (residues 93 to 101): Q H S R E I P Y T

1F6 CDR-H1 (residues 26 to 35): G Y T F T *N Y G* M N
2F2 CDR-H1 (residues 26 to 35): G Y T F S *T Y W* I E

1F6 CDR-H2 (residues 49 to 66): W I *N T Y* T G *E P T* Y *A* D *A* F K G
2F2 CDR-H2 (residues 49 to 66): E I *L G P* S G *Y T D* Y *N* E *K* F K A

1F6 CDR-H3 (residues 99 to 107): *D Y G D* Y G M D Y
2F2 CDR-H3 (residues 99 to 107): *W D R L* Y A M D Y

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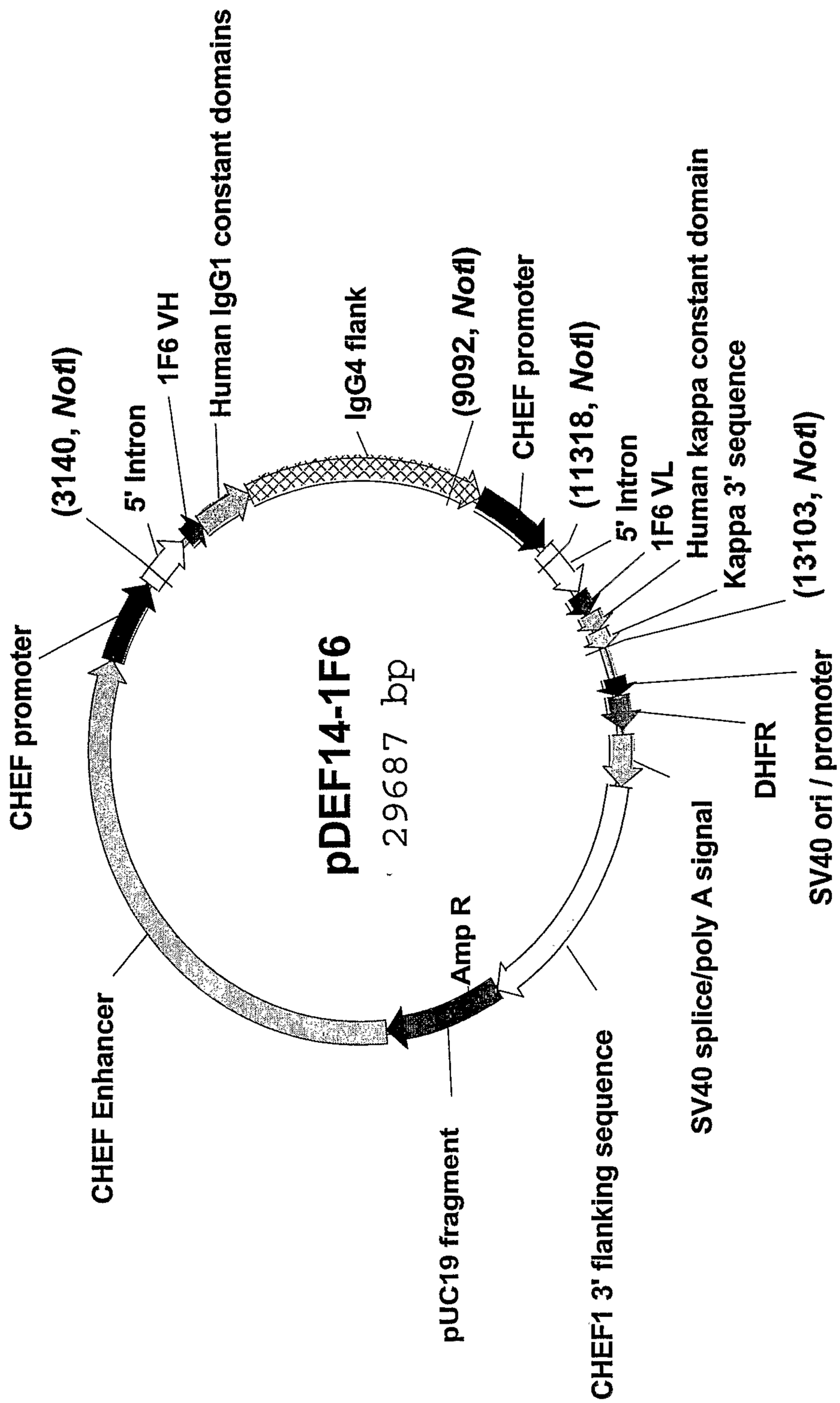
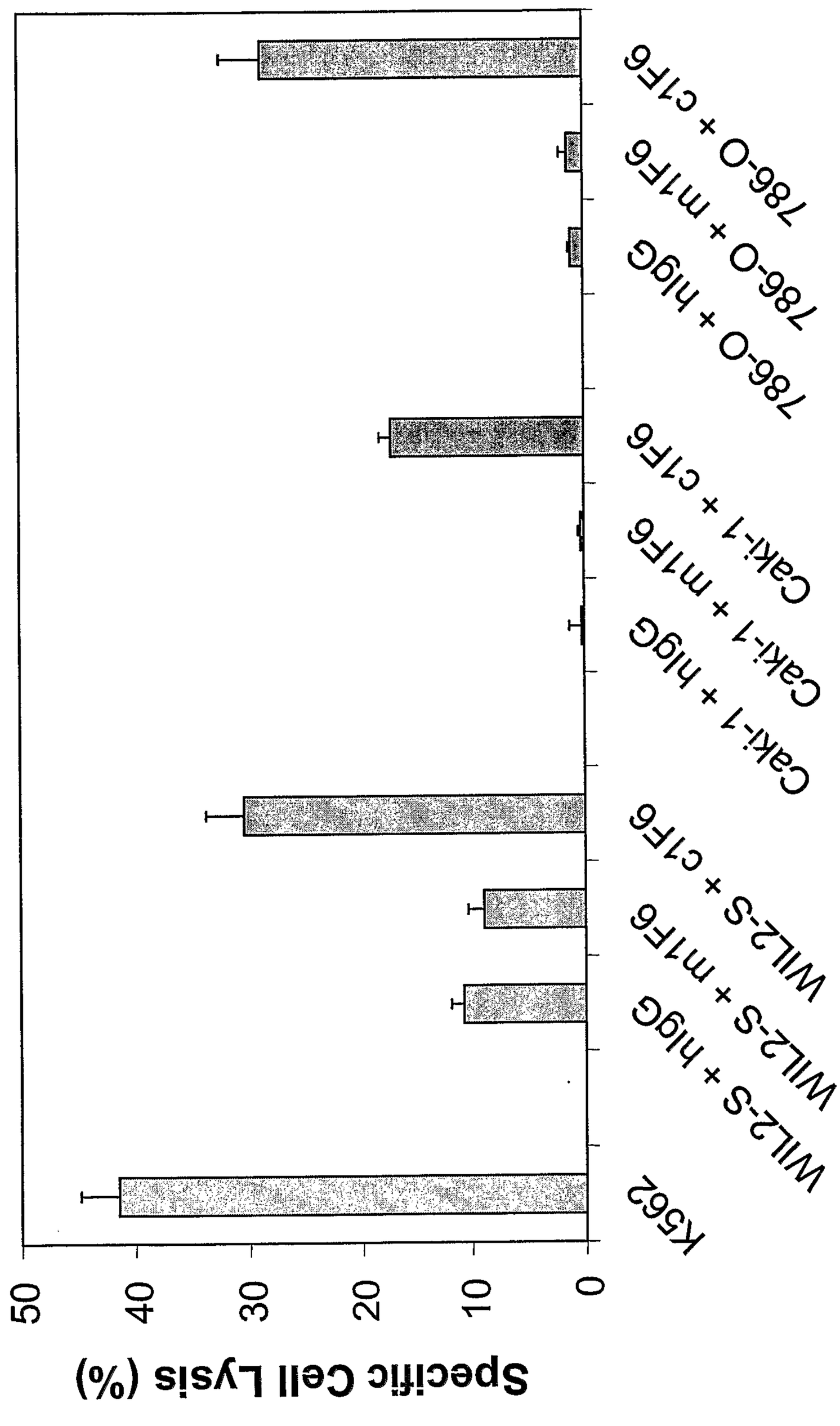

pDEF14-1F6
29687 bp
CHEF promoter
(3140, NotI)
5' Intron
1F6 VH
Human IgG1 constant domains
IgG4 flank
(9092, NotI)
CHEF promoter
(11318, NotI)
5' Intron
1F6 VL
Human kappa constant domain
Kappa 3' sequence
(13103, NotI)
SV40 ori / promoter
DHFR
SV40 splice/poly A signal
CHEF1 3' flanking sequence
pUC19 fragment
Amp R
CHEF Enhancer

Figure 4

Figure 5


Specific Cell Lysis (%)
50
40
30
20
10
0
K562
WIL2-S + hIgG
WIL2-S + m1FE6
WIL2-S + c1FE6
Caki-1 + hIgG
Caki-1 + m1FE6
Caki-1 + c1FE6
786-O + hIgG
786-O + m1FE6
786-O + c1FE6

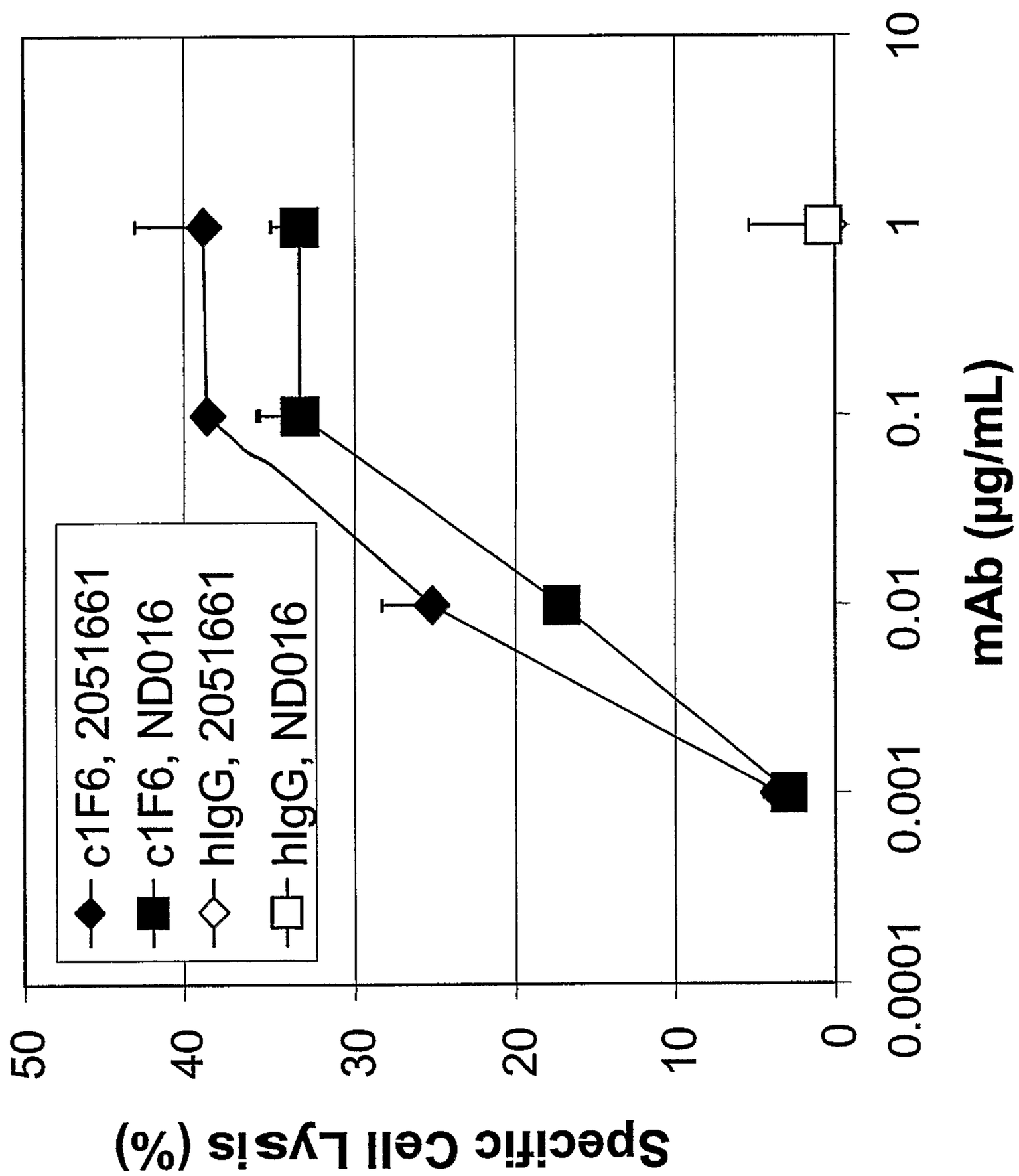

c1F6, 2051661
c1F6, ND016
hIgG, 2051661
hIgG, ND016
Specific Cell Lysis (%)
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
mAb (µg/mL)

Figure 6

Figure 7

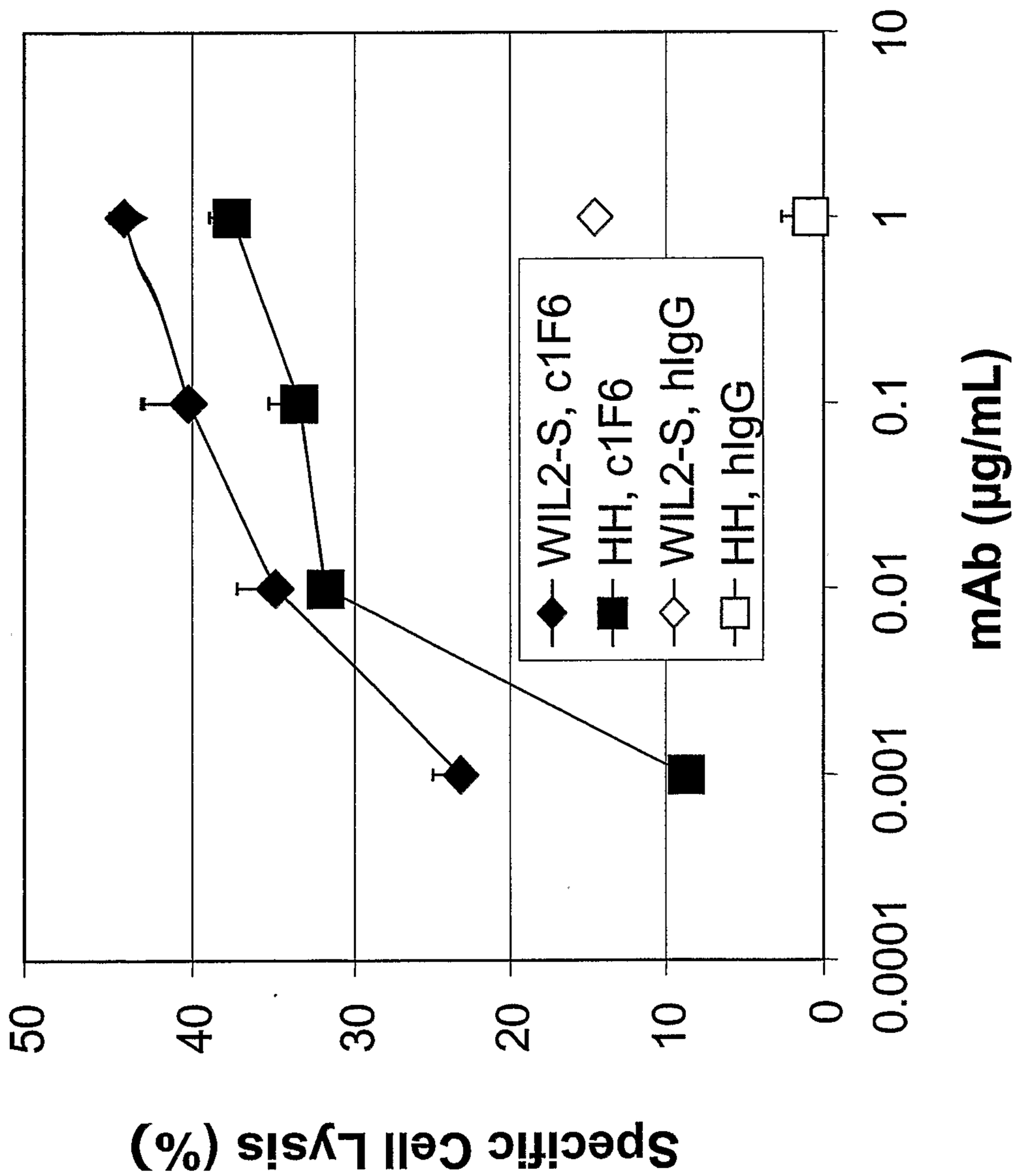
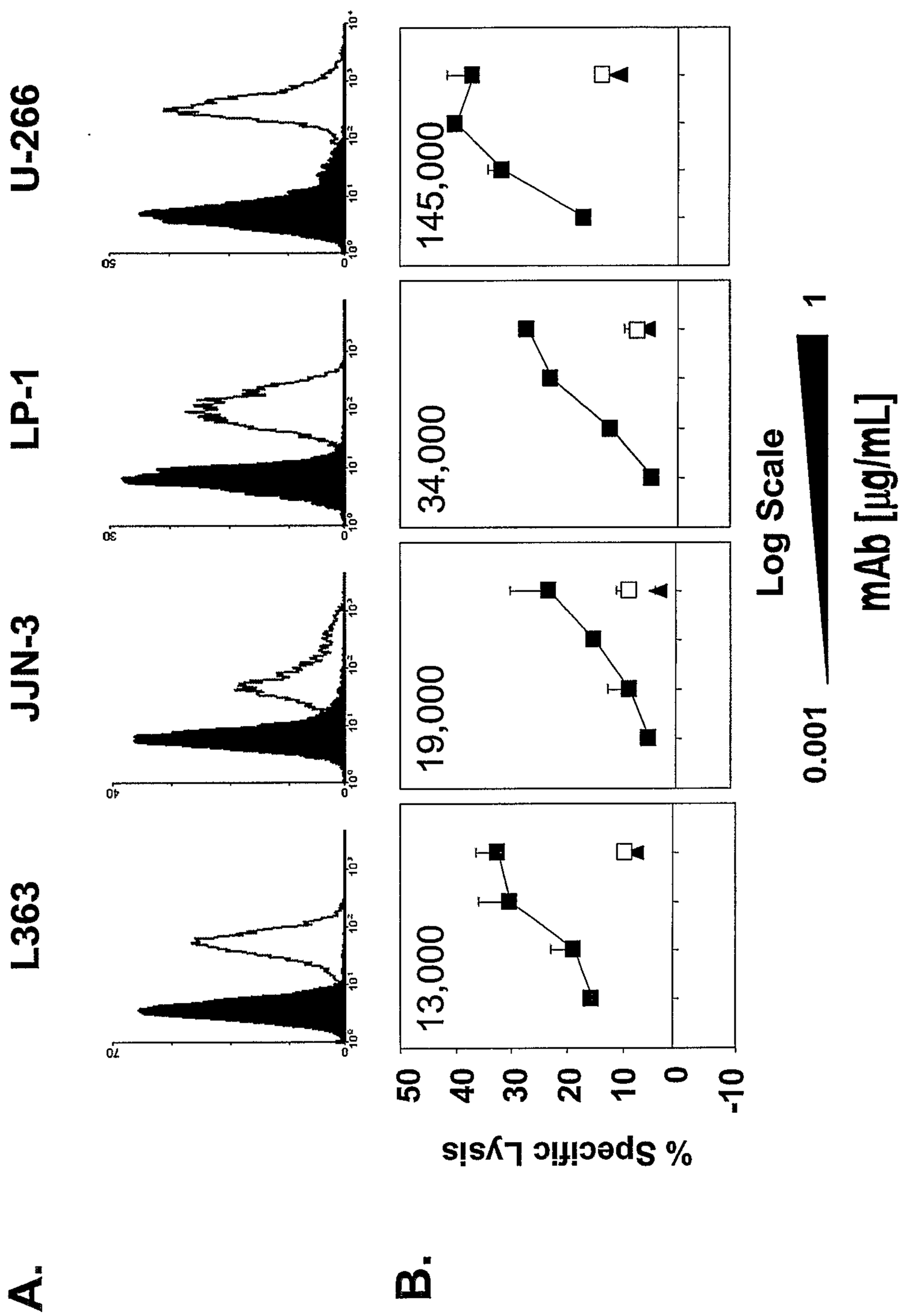

Specific Cell Lysis (%)
50
40
30
20
10
0
0.0001
0.001
0.01
0.1
1
10
mAb (µg/mL)
WIL2-S, c1F6
HH, c1F6
WIL2-S, hIgG
HH, hIgG

Figure 8


A.
L363
JJN-3
LP-1
U-266
B.
13,000
19,000
34,000
145,000
% Specific Lysis
50
40
30
20
10
0
-10
Log Scale
0.001
1
mAb [µg/mL]

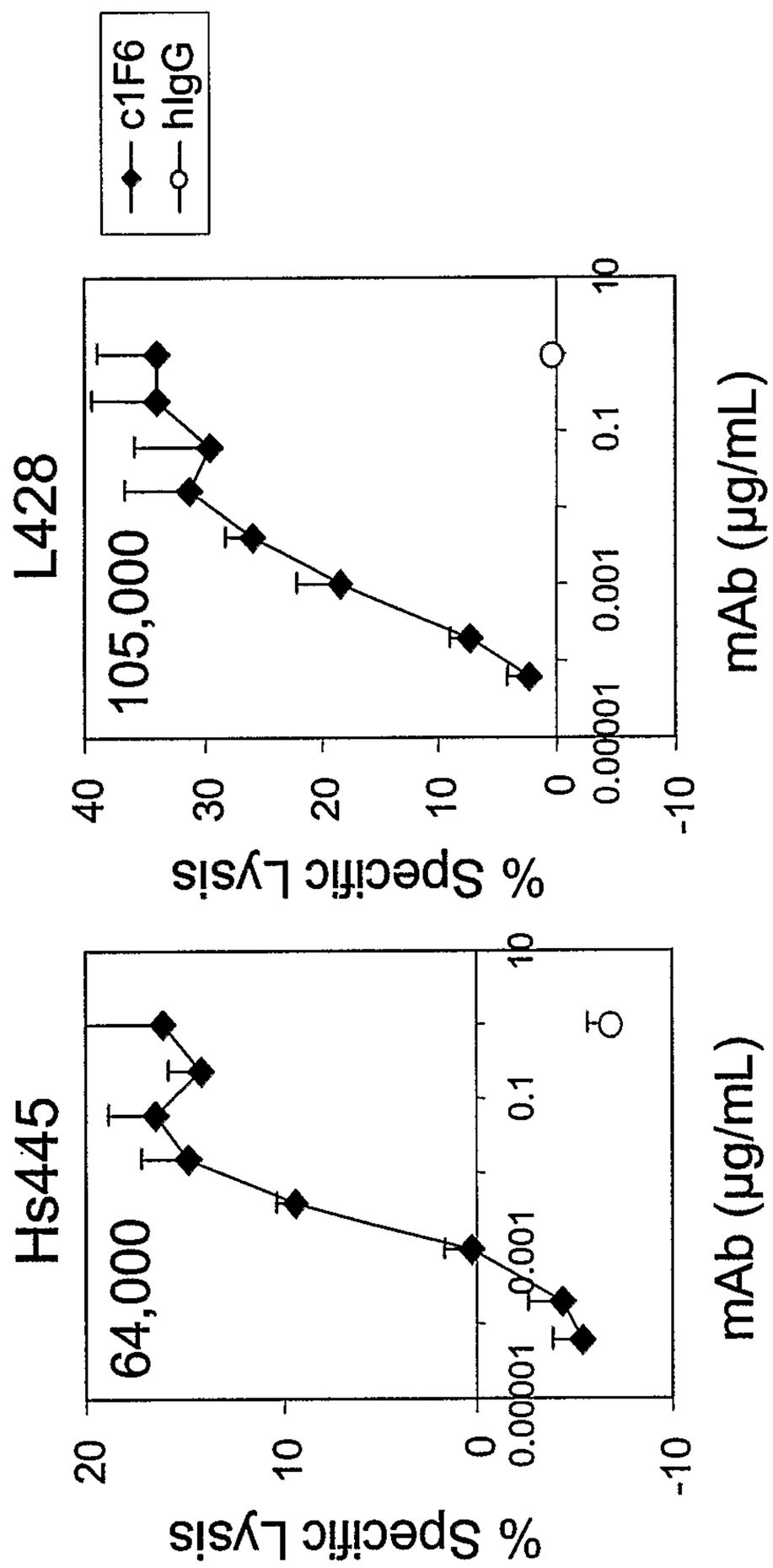

c1F6
hIgG
L428
105,000
% Specific Lysis
40
30
20
10
0
-10
0.0001
0.001
0.1
10
mAb (µg/mL)
Hs445
64,000
% Specific Lysis
20
10
0
-10
0.00001
0.001
0.1
10
mAb (µg/mL)

Figure 9

Figure 10

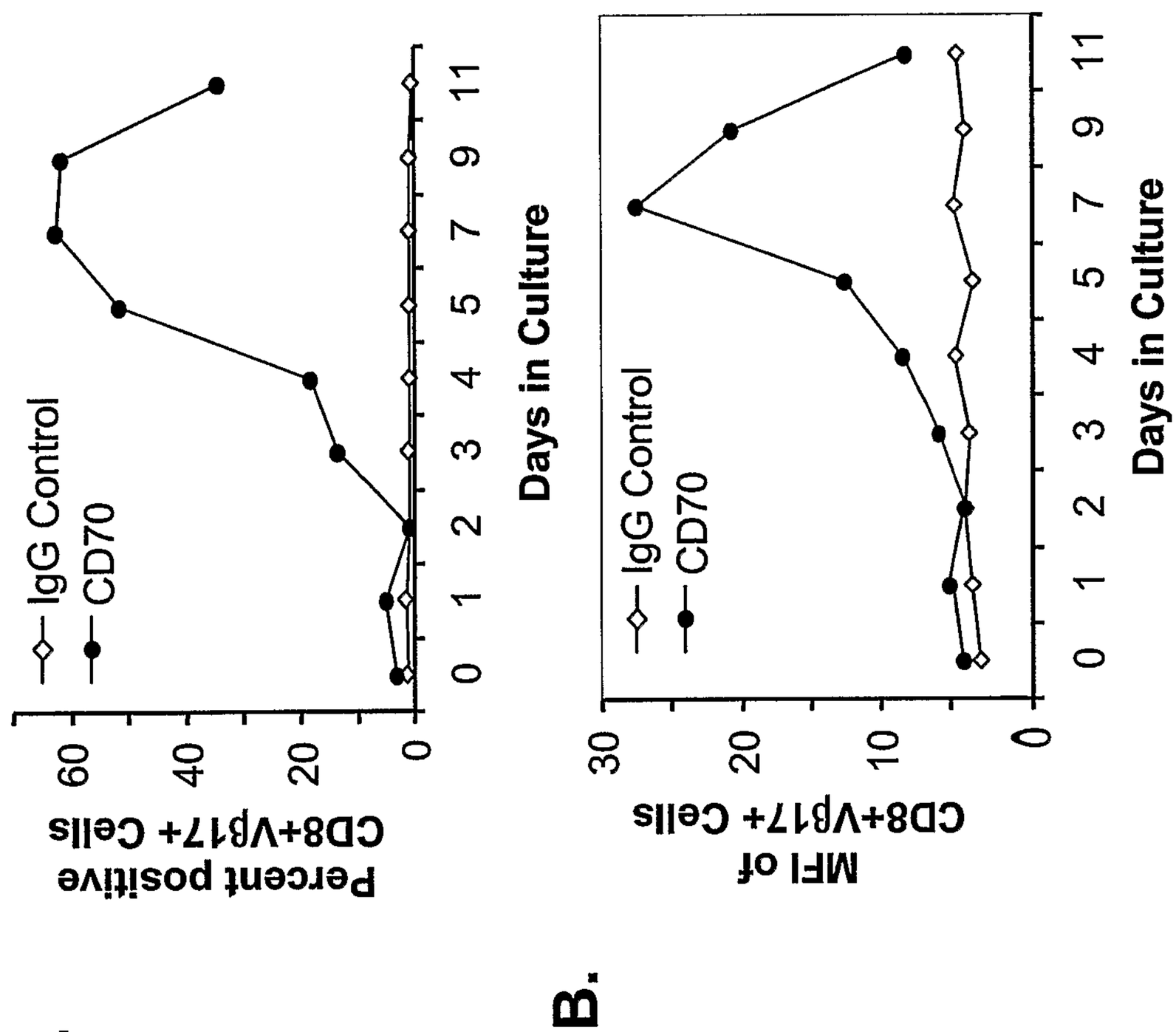

A.
IgG Control
CD70
Percent positive CD8+Vβ17+ Cells
60
40
20
0
0 1 2 3 4 5 7 9 11
Days in Culture
B.
IgG Control
CD70
MFI of CD8+Vβ17+ Cells
30
20
10
0
0 1 2 3 4 5 7 9 11
Days in Culture

Figure 11

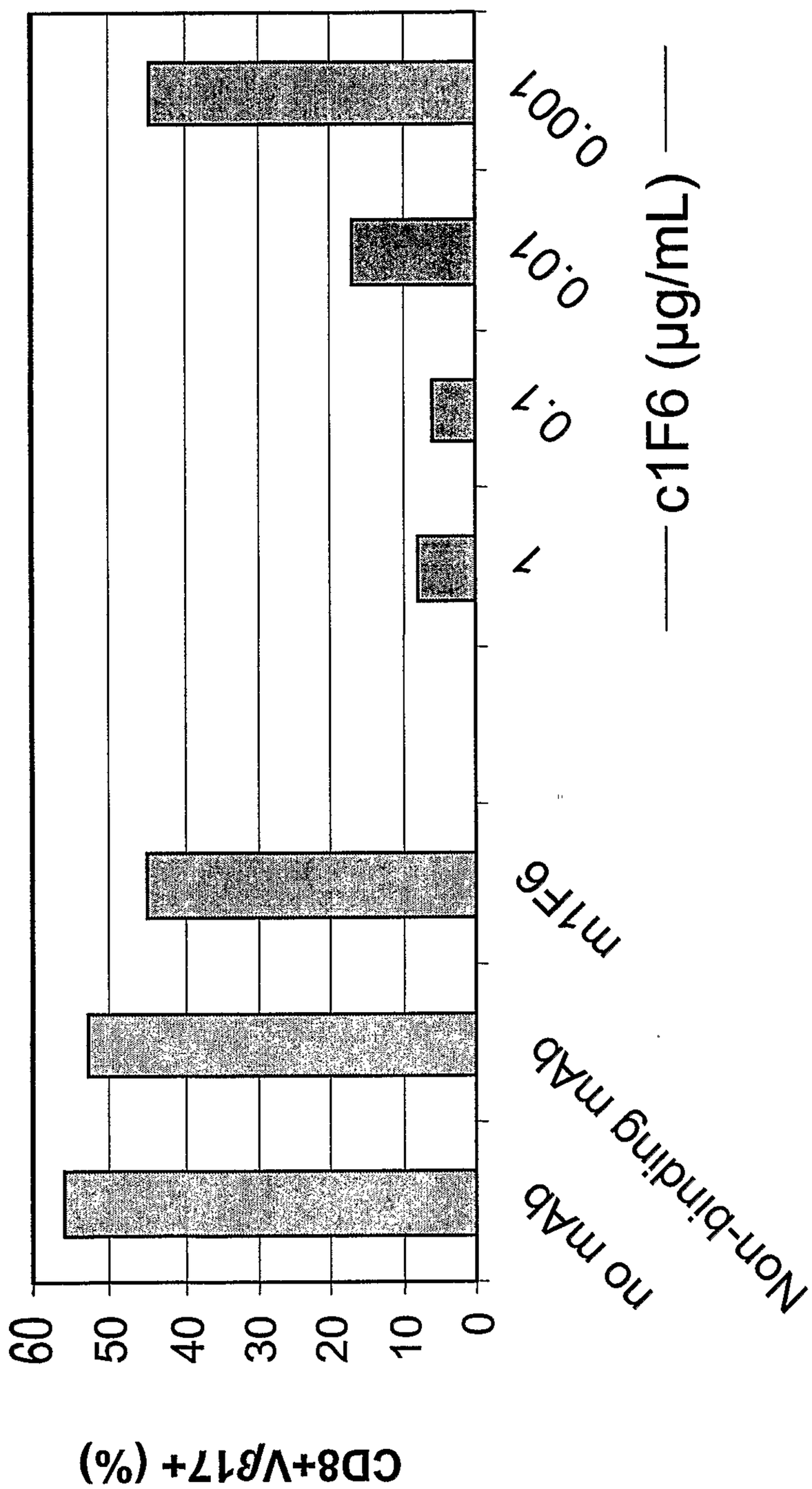
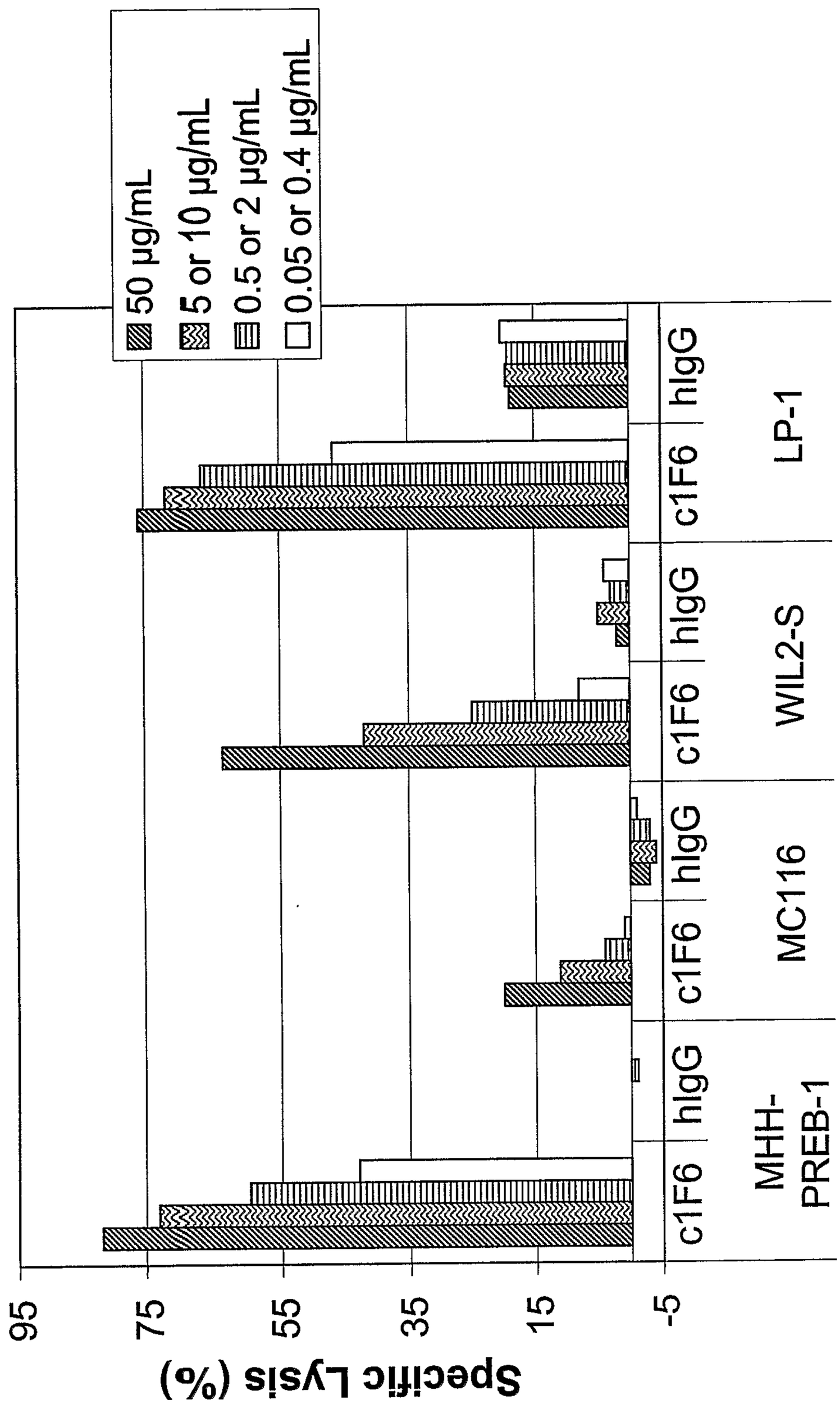

CD8+Vβ17+ (%)
60
50
40
30
20
10
0
no mAb
Non-binding mAb
m1F6
1
0.1
0.01
0.001
c1F6 (μg/mL)

Figure 12


50 µg/mL
5 or 10 µg/mL
0.5 or 2 µg/mL
0.05 or 0.4 µg/mL
Specific Lysis (%)
95
75
55
35
15
-5
c1F6
hIgG
MHH-PREB-1
MC116
WIL2-S
LP-1

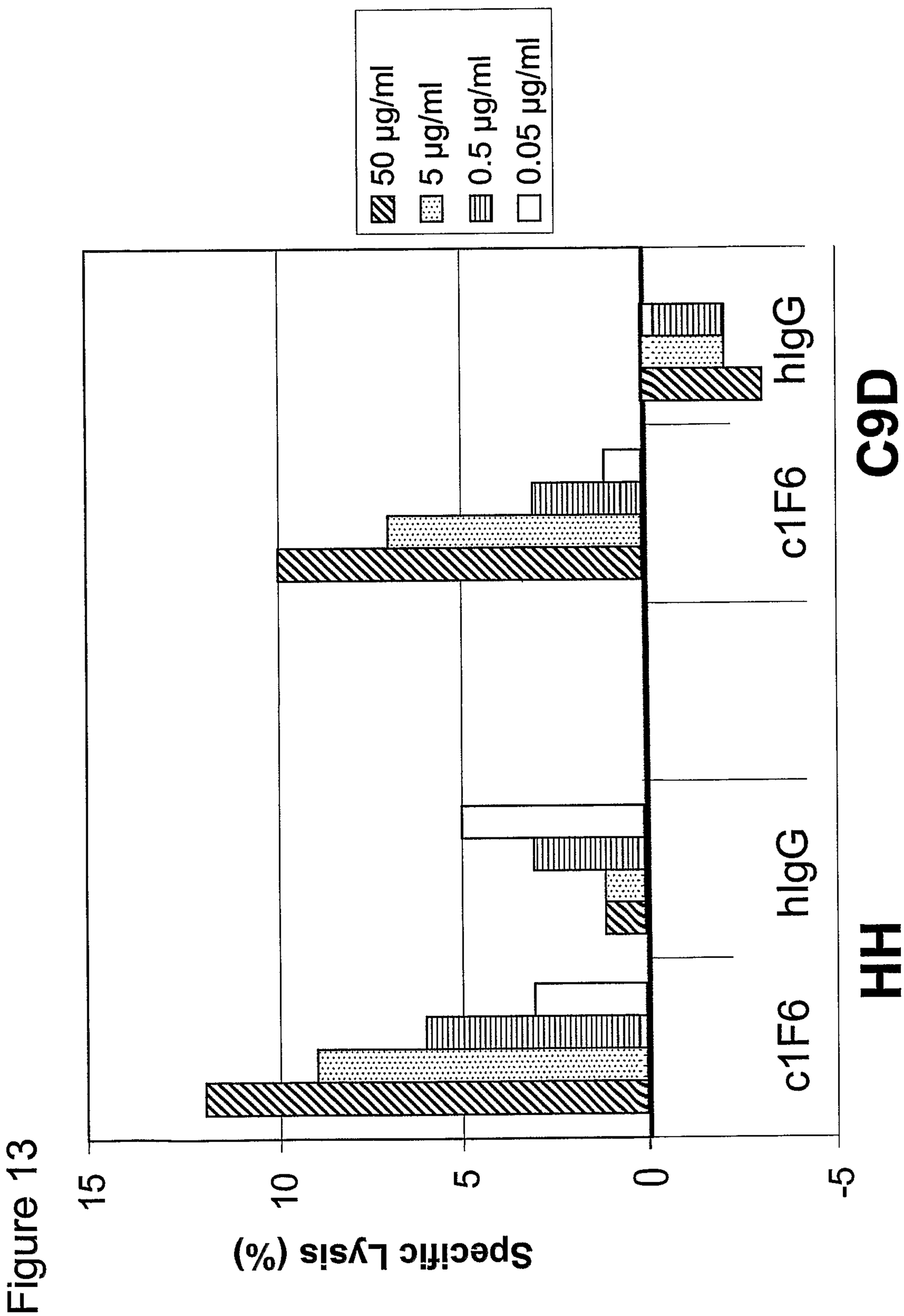
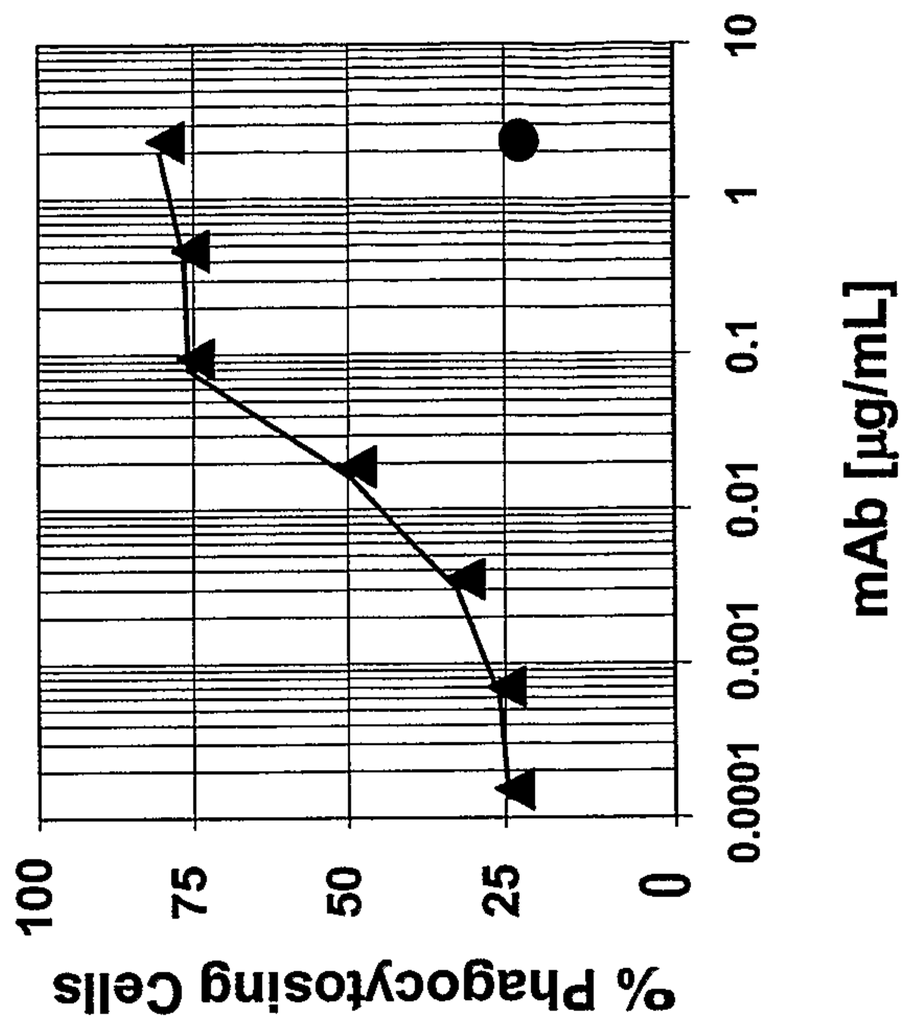

50 µg/ml
5 µg/ml
0.5 µg/ml
0.05 µg/ml
Specific Lysis (%)
15
10
5
0
-5
c1F6
hIgG
c1F6
hIgG
HH
C9D

Figure 13


% Phagocytosing Cells
100
75
50
25
0
0.0001
0.001
0.01
0.1
1
10
mAb [µg/mL]

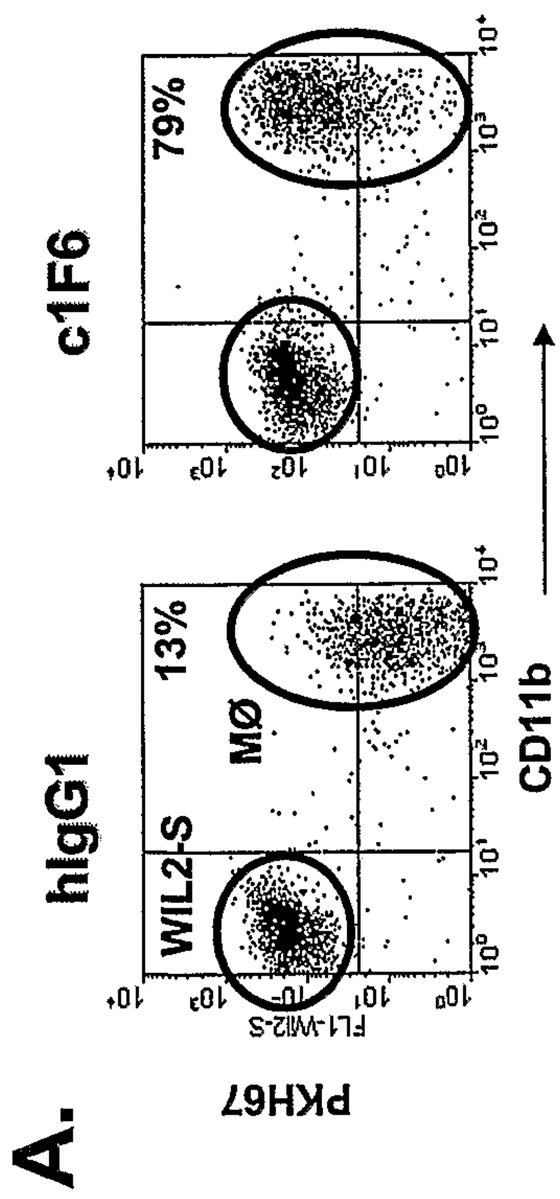

A.
hIgG1
WIL2-S
MØ
13%
79%
PKH67
FL1-WIL2-S
CD11b
B.

Figure 14

Figure 15

Cell Line	% specific ADCP activity
Renal Cell Carcinoma	
786-O	62
A498	55
Hodgkin's Disease	
KMH2	8
L428	25
Hs445	38
RPMI-6666	49
Multiple Myeloma	
LP-1	30
Non-Hodgkin's Lymphoma	
MHH-PreB-1	45
B cell lymphoma	
WIL2-S	39

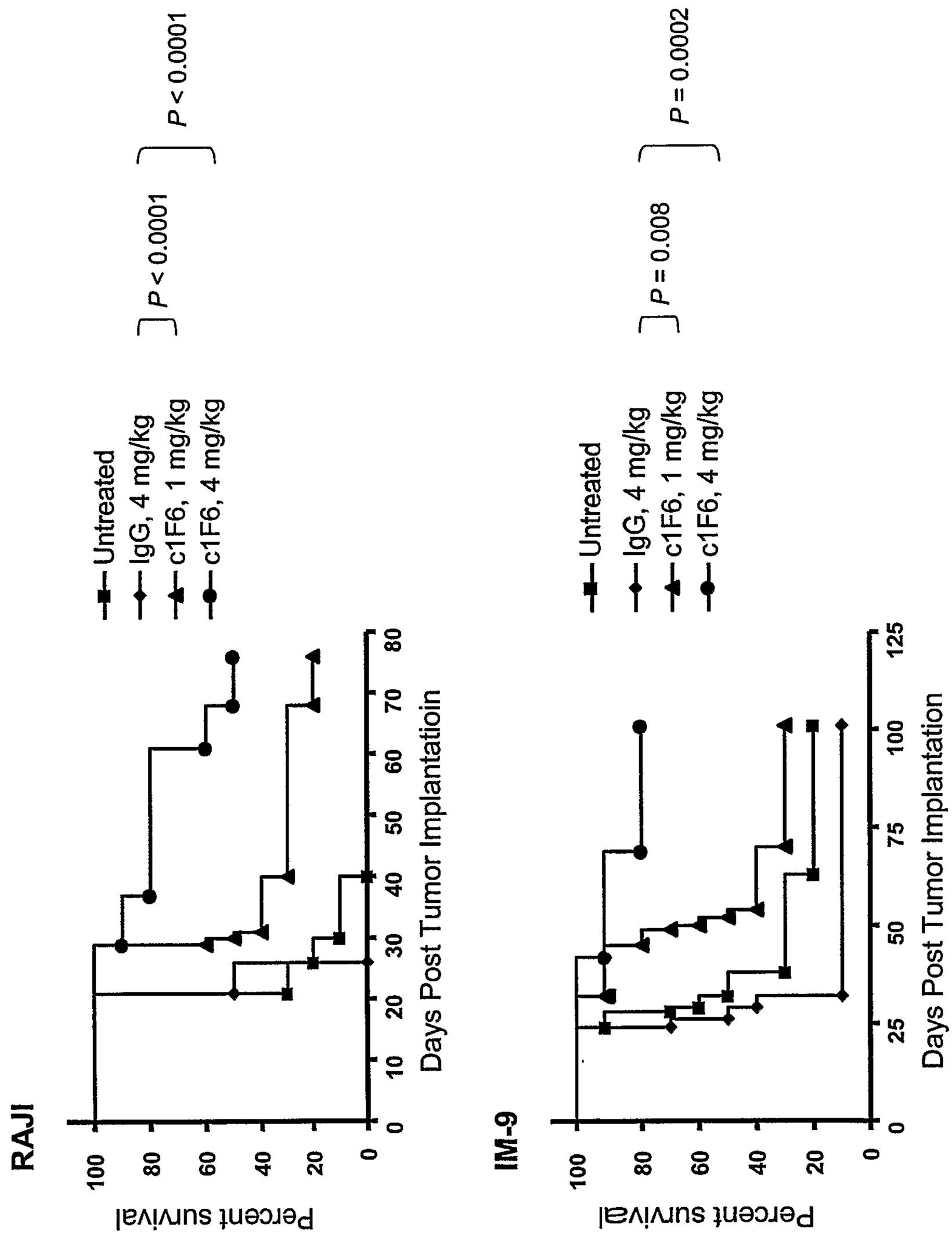

RAJI
Untreated
IgG, 4 mg/kg
c1F6, 1 mg/kg
c1F6, 4 mg/kg
P < 0.0001
P < 0.0001
Percent survival
Days Post Tumor Implantatioin
IM-9
Untreated
IgG, 4 mg/kg
c1F6, 1 mg/kg
c1F6, 4 mg/kg
P = 0.0002
P = 0.008
Percent survival
Days Post Tumor Implantation

Figure 16