ANTIBODIES TO M-CSF

[0001] This application claims the benefit of United States Provisional Application 60/502,163, filed September 10, 2003.

Background of the Invention

[0002] Macrophage colony stimulating factor (M-CSF) is a member of the family of proteins referred to as colony stimulating factors (CSFs). M-CSF is a secreted or a cell surface glycoprotein comprised of two subunits that are joined by a disulfide bond with a total molecular mass varying from 40 to 90 kD ((Stanley E.R., *et al.*, *Mol. Reprod. Dev.*, 46:4-10 (1997)). Similar to other CSFs, M-CSF is produced by macrophages, monocytes, and human joint tissue cells, such as chondrocytes and synovial fibroblasts, in response to proteins such as interleukin-1 or tumor necrosis factor-alpha. M-CSF stimulates the formation of macrophage colonies from pluripotent hematopoietic progenitor stem cells (Stanley E.R., *et al.*, *Mol. Reprod. Dev.*, 46:4-10 (1997)).

[0003] M-CSF typically bind to its receptor, *c-fms*, in order to exert a biological effect. *c-fms* contains five extracellular Ig domains, one transmembrane domain, and an intracellular domain with two kinase domains. Upon M-CSF binding to *c-fms*, the receptor homo-dimerizes and initiates a cascade of signal transduction pathways including the JAK/STAT, PI3K, and ERK pathways.

[0004] M-CSF is an important regulator of the function, activation, and survival of monocytes/macrophages. A number of animal models have confirmed the role of M-CSF in various diseases, including rheumatoid arthritis (RA) and cancer. Macrophages comprise key effector cells in RA. The degree of synovial

macrophage infiltration in RA has been shown to closely correlate with the extent of underlying joint destruction. M-CSF, endogenously produced in the rheumatoid joint by monocytes/macrophages, fibroblasts, and endothelial cells, acts on cells of the monocyte/macrophage lineage to promote their survival and differentiation into bone destroying osteoclasts, and enhance pro-inflammatory cellular functions such as cytotoxicity, superoxide production, phagocytosis, chemotaxis and secondary cytokine production. For example, treatment with M-CSF in the rat streptococcus agalactiae sonicate-induced experimental arthritis model lead to enhanced pathology (Abd, A.H., *et al.*, *Lymphokine Cytokine Res.* 10:43-50 (1991)). Similarly, subcutaneous injections of M-CSF in a murine model of collagen-induced arthritis (CIA), which is a model for RA, resulted in a significant exacerbation of the RA disease symptoms (Campbell I.K., *et al.*, *J. Leuk. Biol.* 68:144-150 (2000)). Furthermore, MRL/lpr mice that are highly susceptible to RA and other autoimmune diseases have elevated basal M-CSF serum concentrations (Yui M.A., *et al.*, *Am. J. Pathol.* 139:255-261 (1991)). The requirement for endogenous M-CSF in maintaining CIA was demonstrated by a significant reduction in the severity of established disease by M-CSF neutralizing mouse monoclonal antibody (Campbell I.K., *et al.*, *J. Leuk. Biol.* 68:144-150 (2000)).

[0005] With respect to cancer, inhibition of colony stimulating factors by antisense oligonucleotides suppresses tumor growth in embryonic and colon tumor xenografts in mice by decelerating macrophage-mediated ECM breakdown (Seyedhossein, A., *et al.*, *Cancer Research*, 62:5317-5324 (2002)).

[0006] M-CSF binding to *c-fms* and its subsequent activation of monocyte/macrophages is important in a number of disease states. In addition to RA and cancer, the other examples of M-CSF-related disease states include osteoporosis, destructive arthritis, atherogenesis, glomerulonephritis, Kawasaki disease, and HIV-1 infection, in which monocytes/macrophages and related cell types play a role. For instance, osteoclasts are similar to macrophages and are regulated in part by M-CSF. Growth and differentiation signals induced by M-CSF in the initial stages of osteoclast maturation are essential for their subsequent osteoclastic activity in bone.

[0007] Osteoclast mediated bone loss, in the form of both focal bone erosions and more diffuse juxta-articular osteoporosis, is a major unsolved problem in RA. The consequences of this bone loss include joint deformities, functional disability, increased risk of bone fractures and increased mortality. M-CSF is uniquely essential for osteoclastogenesis and experimental blockade of this cytokine in animal models of arthritis successfully abrogates joint destruction. Similar destructive pathways are known to operate in other forms of destructive arthritis such as psoriatic arthritis, and could represent venues for similar intervention.

[0008] Postmenopausal bone loss results from defective bone remodeling secondary to an uncoupling of bone formation from exuberant osteoclast mediated bone resorption as a consequence of estrogen deficiency. *In-vivo* neutralization of M-CSF using a blocking antibody has been shown in mice to completely prevent the rise in osteoclast numbers, the increase in bone resorption and the resulting bone loss induced by ovariectomy.

[0009] Several lines of evidence point to a central role for M-CSF in atherogenesis, and in proliferative intimal hyperplasia after mechanical trauma to the arterial wall. All the major cell types in atherosclerotic lesions have been shown to express M-CSF, and this is further up-regulated by exposure to oxidized lipoprotein. Blockade of M-CSF signaling with a neutralizing *c-fms* antibody reduces the accumulation of macrophage-derived foam cells in the aortic root of apolipoprotein E deficient mice maintained on a high fat diet.

[0010] In both experimental and human glomerulonephritis, glomerular M-CSF expression has been found to co-localize with local macrophage accumulation, activation and proliferation and correlate with the extent of glomerular injury and proteinuria. Blockade of M-CSF signaling via an antibody directed against its receptor *c-fms* significantly down-regulates local macrophage accumulation in mice during the renal inflammatory response induced by experimental unilateral ureteric obstruction.

[0011] Kawasaki disease (KD) is an acute, febrile, pediatric vasculitis of unknown cause. Its most common and serious complications involve the coronary vasculature in the form of aneurismal dilatation. Serum M-CSF levels are significantly elevated in acute phase Kawasaki's disease, and normalize following

treatment with intravenous immunoglobulin. Giant cell arthritis (GCA) is an inflammatory vasculopathy mainly occurring in the elderly in which T cells and macrophages infiltrate the walls of medium and large arteries leading to clinical consequences that include blindness and stroke secondary to arterial occlusion. The active involvement of macrophages in GCA is evidenced by the presence of elevated levels of macrophage derived inflammatory mediators within vascular lesions.

[0012] M-CSF has been reported to render human monocyte derived macrophages more susceptible to HIV-1 infection *in vitro*. In a recent study, M-CSF increased the frequency with which monocyte-derived macrophages became infected, the amount of HIV mRNA expressed per infected cell, and the level of proviral DNA expressed per infected culture.

[0013] Given the role of M-CSF in various diseases, a method for inhibiting M-CSF activity is desirable.

[0014] There is a critical need for therapeutic anti-M-CSF antibodies.

SUMMARY OF THE INVENTION

[0015] The present invention provides isolated human antibodies or antigen-binding portions thereof that specifically bind human M-CSF and acts as a M-CSF antagonist and compositions comprising said antibody or portion.

[0016] The invention also provides for compositions comprising the heavy and/or light chain, the variable regions thereof, or antigen-binding portions thereof an anti-M-CSF antibody, or nucleic acid molecules encoding an antibody, antibody chain or variable region thereof the invention effective in such treatment and a pharmaceutically acceptable carrier. In certain embodiments, the compositions may further comprise another component, such as a therapeutic agent or a diagnostic agent. Diagnostic and therapeutic methods are also provided by the invention. In certain embodiments, the compositions are used in a therapeutically effective amount necessary to treat or prevent a particular disease or condition.

[0017] The invention also provides methods for treating or preventing a variety of diseases and conditions such as, but not limited to, inflammation, cancer, atherogenesis, neurological disorders and cardiac disorders with an effective amount of an anti-M-CSF antibody of the invention, or antigen binding portion thereof, nucleic acids encoding said antibody, or heavy and/or light chain, the variable regions, or antigen-binding portions thereof.

[0018] The invention provides isolated cell lines, such as a hybridomas, that produce anti-M-CSF antibodies or antigen-binding portions thereof.

[0019] The invention also provides nucleic acid molecules encoding the heavy and/or light chains of anti-M-CSF antibodies, the variable regions thereof, or the antigen-binding portions thereof.

[0020] The invention provides vectors and host cells comprising the nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules.

[0021] Non-human transgenic animals or plants that express the heavy and/or light chains, or antigen-binding portions thereof, of anti-M-CSF antibodies are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A and 1B are graphs illustrating that the anti-M-CSF antibodies resulted in a dose-related decrease in total monocyte counts in male and female monkeys over time. The monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system. Monocyte counts were monitored from 24 hours through 3 weeks after administration of vehicle or antibody 8.10.3 at 0, 0.1, 1 or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period.

[0023] Figure 1A male monkeys.

[0024] Figure 1B female monkeys.

[0025] Figures 2A and 2B are graphs illustrating that anti-M-CSF treatment resulted in a reduction in the percentage of CD14+CD16+ monocytes, in male and female monkeys. 0-21 days after administration of vehicle or antibody 8.10.3 at 0, 0.1, 1 or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. For each monkey tested, the percentage of monocytes within the CD14+CD16+ subset was determined after each blood draw, on days 1, 3, 7, 14 and 21 after 8.10.3 injection.

[0026] Figure 2A male monkeys.

[0027] Figure 2B female monkeys.

[0028] Figures 3A and 3B are graphs illustrating that anti-M-CSF treatment resulted in a decrease in the percentage change of total monocytes at all doses of antibody 8.10.3F and antibody 9.14.4I as compared to pre-test levels of monocytes.

[0029] Figure 3A shows data collected from experiments using antibody 8.10.3F.

[0030] Figure 3B shows data collected from experiments using antibody 9.14.4I.

[0031] Figure 4 is a sequence alignment of the predicted amino acid sequences of light and heavy chain variable regions from twenty-six anti-M-CSF antibodies compared with the germline amino acid sequences of the corresponding variable region genes. Differences between the antibody sequences and the germline gene sequences are indicated in bold-faced type. Dashes represent no change from germline. The underlined sequences in each alignment represent, from left to right, the FR1, CDR1, FR2, CDR2, FR3, CDR3 AND FR4 sequences.

[0032] Figure 4A shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 252 (residues 21-127 of SEQ ID NO: 4) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0033] Figure 4B shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 88 (residues 21-127 of SEQ ID NO: 8) to the germline $V_\kappa O12$, $J_\kappa 3$ sequence (SEQ ID NO: 103).

[0034] Figure 4C shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 100 (residues 21-127 of SEQ ID NO: 12) to the germline $V_\kappa L2$, $J_\kappa 3$ sequence (SEQ ID NO: 107).

[0035] Figure 4D shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 3.8.3 (residues 23-130 of SEQ ID NO: 16) to the germline $V_\kappa L5$, $J_\kappa 3$ sequence (SEQ ID NO: 109).

[0036] Figure 4E shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 2.7.3 (residues 23-130 of SEQ ID NO: 20) to the germline $V_\kappa L5$, $J_\kappa 4$ sequence (SEQ ID NO: 117).

[0037] Figure 4F shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 1.120.1 (residues 21-134 of SEQ ID NO: 24) to the germline $V_\kappa B3$, $J_\kappa 1$ sequence (SEQ ID NO: 112).

[0038] Figure 4G shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 252 (residues 20-136 of SEQ ID NO: 2) to the germline $V_H 3\text{-}11$, $D_H 7\text{-}27$ $J_H 6$ sequence (SEQ ID NO: 106).

[0039] Figure 4H shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 88 (residues 20-138 of SEQ ID NO: 6) to the germline $V_H 3\text{-}7$, $D_H 6\text{-}13$, $J_H 4$ sequence (SEQ ID NO: 105).

[0040] Figure 4I shows the alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 100 (residues 20-141 of SEQ ID NO: 10) to the germline $V_H 3\text{-}23$, $D_H 1\text{-}26$, $J_H 4$ sequence (SEQ ID NO: 104).

[0041] Figure 4J shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 3.8.3 (residues 20-135 of SEQ ID NO: 14) to the germline $V_H 3\text{-}11$, $D_H 7\text{-}27$, $J_H 4$ sequence (SEQ ID NO: 108).

[0042] Figure 4K shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 2.7.3 (residues 20-137 of SEQ ID NO: 18) to the germline V_H3-33, D_H1-26, J_H4 sequence (SEQ ID NO: 110).

[0043] Figure 4L shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 1.120.1 (residues 20-139 of SEQ ID NO: 22) to the germline V_H1-18, D_H4-23, J_H4 sequence (SEQ ID NO: 111).

[0044] Figure 4M shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3 (residues 21-129 of SEQ ID NO: 44) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0045] Figure 4N shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3 (residues 20-141 of SEQ ID NO: 30) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0046] Figure 4O shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4 (residues 23-130 of SEQ ID NO: 28) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0047] Figure 4P shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4 (residues 20-135 of SEQ ID NO: 38) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0048] Figure 4Q shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2 (residues 23-130 of SEQ ID NO: 48) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0049] Figure 4R shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2 (residues 20-136 of SEQ ID NO: 46) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0050] Figure 4S shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4I (residues 23-130 of SEQ ID NO: 28) to the germline V_κO12 J_κ3 sequence (SEQ ID NO: 103).

[0051] Figure 4T shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4I (residues 20-135 of SEQ ID NO: 26) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0052] Figure 4U shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3F (residues 21-129 of SEQ ID NO: 32) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0053] Figure 4V shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3F (residues 20-141 of SEQ ID NO: 30) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0054] Figure 4W shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2IF (residues 23-130 of SEQ ID NO: 36) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0055] Figure 4X shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2IF (residues 20-136 of SEQ ID NO: 34) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0056] Figure 4Y shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2C-Ser (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0057] Figure 4Z shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2C-Ser (residues 20-136 of SEQ ID NO: 50) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0058] Figure 4AA shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4C-Ser (residues 23-130 of SEQ ID NO: 56) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0059] Figure 4BB shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4C-Ser (residues 20-135 of SEQ ID NO: 54) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0060] Figure 4CC shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3C-Ser (residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0061] Figure 4DD shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3C-Ser (residues 20-141 of SEQ ID NO: 58) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0062] Figure 4EE shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-CG2 (residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0063] Figure 4FF shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-CG2 (residues 20-141 of SEQ ID NO: 62) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0064] Figure 4GG shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-CG2 (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0065] Figure 4HH shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-CG2 (residues 20-136 of SEQ ID NO: 66) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0066] Figure 4II shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-CG4 (residues 23-130 of SEQ ID NO: 52) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0067] Figure 4JJ shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-CG4 (residues 20-135 of SEQ ID NO: 70) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0068] Figure 4KK shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-CG2 (residues 23-130 of SEQ ID NO: 56) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0069] Figure 4LL shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-CG2 (residues 20-135 of SEQ ID NO: 74) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0070] Figure 4MM shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-CG4 (residues 23-130 of SEQ ID NO: 56) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0071] Figure 4NN shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-CG4 (residues 20-135 of SEQ ID NO: 78) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0072] Figure 4OO shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4-Ser (residues 23-130 of SEQ ID NO: 28) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0073] Figure 4PP shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4-Ser (residues 20-135 of SEQ ID NO: 82) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0074] Figure 4QQ shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.7.2-Ser (residues 23-130 of SEQ ID NO: 48) to the germline V_κO12, J_κ3 sequence (SEQ ID NO: 103).

[0075] Figure 4RR shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.7.2-Ser (residues 20-136 of SEQ ID NO: 86) to the germline V_H3-11, D_H6-13, J_H6b sequence (SEQ ID NO: 115).

[0076] Figure 4SS shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-Ser (residues 21-129 of SEQ ID NO: 44) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0077] Figure 4TT shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-Ser (residues 20-141 of SEQ ID NO: 90) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0078] Figure 4UU shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3-CG4 (residues 21-129 of SEQ ID NO: 60) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0079] Figure 4VV shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3-CG4 (residues 20-141 of SEQ ID NO: 94) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

[0080] Figure 4WW shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 9.14.4G1 (residues 23-130 of SEQ ID NO: 28) to the germline V_κO12 J_κ3 sequence (SEQ ID NO: 103).

[0081] Figure 4XX shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 9.14.4G1 (residues 20-135 of SEQ ID NO: 102) to the germline V_H3-11, D_H7-27, J_H4b sequence (SEQ ID NO: 116).

[0082] Figure 4YY shows an alignment of the predicted amino acid sequence of the light chain variable region for antibody 8.10.3FG1 (residues 21-129 of SEQ ID NO:32) to the germline V_κA27, J_κ4 sequence (SEQ ID NO: 114).

[0083] Figure 4ZZ shows an alignment of the predicted amino acid sequence of the heavy chain variable region for antibody 8.10.3FG1 (residues 20-141 of SEQ ID NO: 98) to the germline V_H3-48, D_H1-26, J_H4b sequence (SEQ ID NO: 113).

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

[0084] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

[0085] The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual,* 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used

for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0086] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0087] The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0088] The term "isolated protein", "isolated polypeptide" or "isolated antibody" is a protein, polypeptide or antibody that by virtue of its origin or source of derivation has one to four of the following: (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

[0089] Examples of isolated antibodies include an anti-M-CSF antibody that has been affinity purified using M-CSF, an anti-M-CSF antibody that has been synthesized by a hybridoma or other cell line *in vitro*, and a human anti-M-CSF antibody derived from a transgenic mouse.

[0090] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

[0091] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. In some embodiments, fragments are at least 5, 6, 8 or 10 amino acids long. In other embodiments, the fragments are at least 14, at least 20, at least 50, or at least 70, 80, 90, 100, 150 or 200 amino acids long.

[0092] The term "polypeptide analog" as used herein refers to a polypeptide that comprises a segment that has substantial identity to a portion of an amino acid sequence and that has at least one of the following properties: (1) specific binding to M-CSF under suitable binding conditions, (2) ability to inhibit M-CSF.

[0093] Typically, polypeptide analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the normally-occurring sequence. Analogs typically are at least 20 or 25 amino acids long, preferably at least 50, 60, 70, 80, 90, 100, 150 or 200 amino acids long or longer, and can often be as long as a full-length polypeptide.

[0094] In certain embodiments, amino acid substitutions of the antibody or antigen-binding portion thereof are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, or (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the normally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the normally-occurring sequence, preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts.

[0095] A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence; e.g., a replacement amino acid should not alter the anti-parallel β-sheet that makes up the immunoglobulin binding domain that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence. In general, glycine and proline analogs would not be used in an anti-parallel β-sheet. Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman

and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0096] Non-peptide analogs are commonly used in the pharmaceutical industry as drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics." Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger, *TINS* p.392 (1985); and Evans *et al.*, *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a desired biochemical property or pharmacological activity), such as a human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH_2NH--, --CH_2S--, --CH_2-CH_2--, --CH=CH--(cis and trans), --$COCH_2$--, --$CH(OH)CH_2$--, and --CH_2SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0097] An "antibody" refers to an intact antibody or an antigen-binding portion that competes with the intact antibody for specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining

region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide.

[0098] From N-terminus to C-terminus, both the mature light and heavy chain variable domains comprise the regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987), or Chothia *et al.*, *Nature* 342:878-883 (1989).

[0099] As used herein, an antibody that is referred to by number is the same as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 3.8.3 is the same antibody as one obtained from hybridoma 3.8.3.

[0100] As used herein, a Fd fragment means an antibody fragment that consists of the V_H and C_H 1 domains; an Fv fragment consists of the V_L and V_H domains of a single arm of an antibody; and a dAb fragment (Ward *et al.*, *Nature* 341:544-546 (1989)) consists of a V_H domain.

[0101] In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain. (Bird *et al.*, *Science* 242:423-426 (1988) and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988).) In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See e.g., Holliger P. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. *et al.*, *Structure* 2:1121-1123 (1994).) In some embodiments, one or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to M-CSF. In such embodiments, the CDR(s) may be incorporated as part of

a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0102] In embodiments having one or more binding sites, the binding sites may be identical to one another or may be different.

[0103] As used herein, the term "human antibody" means any antibody in which the variable and constant domain sequences are human sequences. The term encompasses antibodies with sequences derived from human genes, but which have been changed, e.g. to decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc. The term emcompasses such antibodies produced recombinantly in non-human cells, which might impart glycosylation not typical of human cells. These antibodies may be prepared in a variety of ways, as described below.

[0104] The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-M-CSF antibody. In another embodiment, all of the CDRs are derived from a human anti-M-CSF antibody. In another embodiment, the CDRs from more than one human anti-M-CSF antibodies are combined in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-M-CSF antibody, a CDR2 from the light chain of a second human anti-M-CSF antibody and a CDR3 from the light chain of a third human anti-M-CSF antibody, and the CDRs from the heavy chain may be derived from one or more other anti-M-CSF antibodies. Further, the framework regions may be derived from one of the anti-M-CSF antibodies from which one or more of the CDRs are taken or from one or more different human antibodies.

[0105] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation

domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. See Bowie *et al.*, *Science* 253:164 (1991).

[0106] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson U. *et al.*, *Ann. Biol. Clin.* 51:19-26 (1993); Jonsson U. *et al.*, *Biotechniques* 11:620-627 (1991); Jonsson B. *et al.*, *J. Mol. Recognit.* 8:125-131 (1995); and Johnsson B. *et al.*, *Anal. Biochem.* 198:268-277 (1991).

[0107] The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0108] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearally along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq$1 mM, preferably $\leq$100 nM and most preferably $\leq$10 nM. In certain embodiments, the K_D is 1 pM to 500 pM. In other embodiments, the K_D is between 500 pM to 1 μM. In other embodiments, the K_D is between 1 μM to 100 nM. In other embodiments, the K_D is between 100 mM to 10 nM. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this

information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct cross-competition studies to find antibodies that competitively bind with one another, e.g., the antibodies compete for binding to the antigen. A high throughout process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731.

[0109] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

[0110] The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms.

[0111] The term "isolated polynucleotide" as used herein means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin or source of derivation, the "isolated polynucleotide" has one to three of the following: (1) is not associated with all or a portion of a polynucleotides with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0112] The term "oligonucleotide" as used herein includes naturally occurring, and modified nucleotides linked together by naturally occurring and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for primers and probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0113] The term "naturally occurring nucleotides" as used herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" as

used herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche *et al.*, *Nucl. Acids Res.* 14:9081 (1986); Stec *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984); Stein *et al.*, *Nucl. Acids Res.* 16:3209 (1988); Zon *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon *et al.*, *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Patent No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

[0114] "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0115] The term "vector", as used herein, means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded DNA loop into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other embodiments, the vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0116] The term "recombinant host cell" (or simply "host cell"), as used herein, means a cell into which a recombinant expression vector has been introduced. It should be understood that "recombinant host cell" and "host cell" mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0117] The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. "High stringency" or "highly stringent" conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. One example of "high stringency" or "highly stringent" conditions is the incubation of a polynucleotide with another polynucleotide, wherein one polynucleotide may be affixed to a solid surface such as a membrane, in a hybridization buffer of 6X SSPE or SSC, 50% formamide, 5X Denhardt's reagent, 0.5% SDS, 100 μg/ml denatured, fragmented salmon sperm

DNA at a hybridization temperature of 42°C for 12-16 hours, followed by twice washing at 55°C using a wash buffer of 1X SSC, 0.5% SDS. See also Sambrook *et al.*, *supra*, pp. 9.50-9.55.

[0118] The term "percent sequence identity" in the context of nucleic acid sequences means the percent of residues when a first contiguous sequence is compared and aligned for maximum correspondence to a second contiguous sequence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36, 48 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Pearson, *Methods Enzymol.* 266:227-258 (1996); Pearson, *J. Mol. Biol.* 276:71-84 (1998); herein incorporated by reference). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference.

[0119] A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

[0120] The term "percent sequence identity" means a ratio, expressed as a percent of the number of identical residues over the number of residues compared.

[0121] The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

[0122] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, as supplied with the programs, share at least 70%, 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, and more preferably at least 97%, 98% or 99% sequence identity. In certain embodiments, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* 243:307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0123] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256:1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0124] Sequence identity for polypeptides, is typically measured using sequence analysis software. Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters, as specified with the programs, to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. (University of Wisconsin WI) FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters, as supplied with the programs. See, e.g., Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-402 (1997).

[0125] The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

[0126] As used herein, the terms "label" or "labeled" refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable

marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, e.g., a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0127] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Human Anti-M-CSF Antibodies and Characterization Thereof

[0128] In one embodiment, the invention provides humanized anti-M-CSF antibodies. In another embodiment, the invention provides human anti-M-CSF antibodies. In some embodiments, human anti-M-CSF antibodies are produced by immunizing a non-human transgenic animal, e.g., a rodent, whose genome comprises human immunoglobulin genes so that the rodent produces human antibodies.

[0129] An anti-M-CSF antibody of the invention can comprise a human kappa or a human lamda light chain or an amino acid sequence derived therefrom. In some embodiments comprising a kappa light chain, the light chain variable domain (V_L) is encoded in part by a human $V_\kappa O12$, $V_\kappa L2$, $V_\kappa L5$, $V_\kappa A27$ or $V_\kappa B3$ gene and a $J_\kappa 1$, $J_\kappa 2$, $J_\kappa 3$, or $J_\kappa 4$ gene. In particular embodiments of the invention, the light chain variable domain is encoded by V_κ O12/Jκ3, V_κ L2/Jκ3, V_κL5/Jκ3, V_κL5/Jκ4, V_κA27/ Jκ4 or V_κB3/Jκ1 gene.

[0130] In some embodiments, the V_L of the M-CSF antibody comprises one or more amino acid substitutions relative to the germline amino acid sequence. In some embodiments, the V_L of the anti-M-CSF antibody comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions relative to the germline amino acid sequence. In some embodiments, one or more of those substitutions from germline is in the CDR regions of the light chain. In some embodiments, the amino acid substitutions relative to germline are at one or more of the same positions as the substitutions relative to germline in any one or more of the V_L of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. For example, the V_L of the anti-M-CSF antibody may contain one or more amino acid substitutions compared to germline found in the V_L of antibody 88, and other amino acid substitutions compared to germline found in the V_L of antibody 252 which utilizes the same V_K gene as antibody 88. In some embodiments, the amino acid changes are at one or more of the same positions but involve a different mutation than in the reference antibody.

[0131] In some embodiments, amino acid changes relative to germline occur at one or more of the same positions as in any of the V_L of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, but the changes may represent conservative amino acid substitutions at such position(s) relative to the amino acid in the reference antibody. For example, if a particular position in one of these antibodies is changed relative to germline and is

glutamate, one may substitute aspartate at that position. Similarly, if an amino acid substitution compared to germline is serine, one may substitute threonine for serine at that position. Conservative amino acid substitutions are discussed *supra*.

[0132] In some embodiments, the light chain of the human anti-M-CSF antibody comprises the amino acid sequence that is the same as the amino acid sequence of the V_L of antibody 252 (SEQ ID NO: 4), 88 (SEQ ID NO: 8), 100 (SEQ ID NO: 12), 3.8.3 (SEQ ID NO: 16), 2.7.3 (SEQ ID NO: 20), 1.120.1 (SEQ ID NO: 24), 9.14.4I (SEQ ID NO: 28), 8.10.3F (SEQ ID NO: 32), 9.7.2IF (SEQ ID NO: 36), 9.14.4 (SEQ ID NO: 28), 8.10.3 (SEQ ID NO: 44), 9.7.2 (SEQ ID NO: 48), 9.7.2C-Ser (SEQ ID NO: 52), 9.14.4C-Ser (SEQ ID NO: 56), 8.10.3C-Ser (SEQ ID NO: 60), 8.10.3-CG2 (SEQ ID NO: 60), 9.7.2-CG2 (SEQ ID NO: 52), 9.7.2-CG4 (SEQ ID NO: 52), 9.14.4-CG2 (SEQ ID NO: 56), 9.14.4-CG4 (SEQ ID NO: 56), 9.14.4-Ser (SEQ ID NO: 28), 9.7.2-Ser (SEQ ID NO: 48), 8.10.3-Ser (SEQ ID NO: 44), 8.10.3-CG4 (SEQ ID NO: 60) 8.10.3FG1 (SEQ ID NO: 32) or 9.14.4G1 (SEQ ID NO: 28), or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the light chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0133] In some embodiments, the light chain of the anti-M-CSF antibody comprises at least the light chain CDR1, CDR2 or CDR3 of a germline or antibody sequence, as described herein. In another embodiment, the light chain may comprise a CDR1, CDR2 or CDR3 regions of an antibody independently selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or CDR regions each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions. In other embodiments, the light chain of the anti-M-CSF antibody comprises the light chain CDR1, CDR2 or CDR3, each of which are independently selected from the CDR1, CDR2 and CDR3 regions of an antibody having a light chain variable region comprising the amino acid sequence

of the V_L region selected from SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or encoded by a nucleic acid molecule encoding the V_L region selected from SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47. The light chain of the anti-M-CSF antibody may comprise the CDR1, CDR2 and CDR3 regions of an antibody comprising the amino acid sequence of the V_L region selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1 or SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60.

[0134] In some embodiments, the light chain comprises the CDR1, CDR2 and CDR3 regions of antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or said CDR regions each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0135] With regard to the heavy chain, in some embodiments, the variable region of the heavy chain amino acid sequence is encoded in part by a human V_H3-11, V_H3-23, V_H3-7, V_H1-18, V_H3-33, V_H3-48 gene and a J_H4, J_H6, J_H4b, or J_H6b gene. In a particular embodiment of the invention, the heavy chain variable region is encoded by V_H3-11/D_H7-27/J_H6, V_H3-7/D_H6-13/J_H4, V_H3-23/D_H1-26/J_H4, V_H3-11/D_H7-27/J_H4, V_H3-33/D_H1-26/J_H4, V_H1-18/D_H4-23/J_H4, V_H3-11/D_H7-27/J_H4b, V_H3-48/D_H1-26/J_H4b, V_H3-11/D_H6-13/J_H6b, V_H3-11/D_H7-27/J_H4b, V_H3-48/D_H1-6/J_H4b, or V_H3-11/D_H6-13/J_H6b gene. In some embodiments, the V_H of the anti-M-CSF antibody contains one or more amino acid substitutions, deletions or insertions (additions) relative to the germline amino acid sequence. In some embodiments, the variable domain of the heavy chain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 mutations from the germline amino acid sequence. In some embodiments, the mutation(s) are non-conservative substitutions compared to the germline amino acid sequence. In some embodiments, the mutations are in the CDR regions of the heavy chain. In some embodiments, the amino acid changes are made at one or more of the same

positions as the mutations from germline in any one or more of the V_H of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In other embodiments, the amino acid changes are at one or more of the same positions but involve a different mutation than in the reference antibody.

[0136] In some embodiments, the heavy chain comprises an amino acid sequence of the variable domain (V_H) of antibody 252 (SEQ ID NO: 2), 88 (SEQ ID NO: 6), 100 (SEQ ID NO: 10), 3.8.3 (SEQ ID NO: 14), 2.7.3 (SEQ. ID NO: 18), 1.120.1 (SEQ. ID NO: 22), 9.14.4I (SEQ ID NO: 26), 8.10.3F (SEQ ID NO: 30), 9.7.2IF (SEQ ID NO: 34), 9.14.4 (SEQ ID NO: 38), 8.10.3 (SEQ ID NO: 30), 9.7.2 (SEQ ID NO: 46), 9.7.2C-Ser (SEQ ID NO: 50), 9.14.4C-Ser (SEQ ID NO: 54), 8.10.3C-Ser (SEQ ID NO: 58), 8.10.3-CG2 (SEQ ID NO: 62), 9.7.2-CG2 (SEQ ID NO: 66), 9.7.2-CG4 (SEQ ID NO: 70), 9.14.4-CG2 (SEQ ID NO: 74), 9.14.4-CG4 (SEQ ID NO: 78), 9.14.4-Ser (SEQ ID NO: 82), 9.7.2-Ser (SEQ ID NO: 86), 8.10.3-Ser (SEQ ID NO: 90) 8.10.3-CG4 (SEQ ID NO: 94), 8.10.3FG1 (SEQ ID NO: 98) or 9.14.4G1 (SEQ ID NO: 102), or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some embodiments, the heavy chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0137] In some embodiments, the heavy chain comprises the heavy chain CDR1, CDR2 and CDR3 regions of antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or said CDR regions each having less than 8, less than 6, less than 4, or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0138] In some embodiments, the heavy chain comprises a germline or antibody CDR3, as described above, of an antibody sequence as described herein, and may

also comprise the CDR1 and CDR2 regions of a germline sequence, or may comprise a CDR1 and CDR2 of an antibody sequence, each of which are independently selected from an antibody comprising a heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another embodiment, the heavy chain comprises a CDR3 of an antibody sequence as described herein, and may also comprise the CDR1 and CDR2 regions, each of which are independently selected from a CDR1 and CDR2 region of a heavy chain variable region comprising an amino acid sequence of the V_H region selected from SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or encoded by a nucleic acid sequence encoding the V_H region selected from SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In another embodiment, the antibody comprises a light chain as disclosed above and a heavy chain as disclosed above.

[0139] One type of amino acid substitution that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. In one embodiment, there is a substitution of a non-canonical cysteine. The substitution can be in a framework region of a variable domain or in the constant domain of an antibody. In another embodiment, the cysteine is in a non-canonical region of the antibody.

[0140] Another type of amino acid substitution that may be made is to remove any potential proteolytic sites in the antibody, particularly those that are in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of any heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is elimination of asparagine-glycine pairs, which form potential deamidation sites, by altering one or both of the residues.

[0141] In some embodiments, the C-terminal lysine of the heavy chain of the anti-M-CSF antibody of the invention is not present (Lewis D.A., *et al., Anal. Chem*, 66(5): 585-95 (1994)). In various embodiments of the invention, the heavy

and light chains of the anti-M-CSF antibodies may optionally include a signal sequence.

[0142] In one aspect, the invention relates to inhibiting human anti-M-CSF monoclonal antibodies and the cell lines engineered to produce them. Table 1 lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that encode the variable region of the heavy and light chains and the corresponding predicted amino acid sequences for the monoclonal antibodies: 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3 and 9.7.2. Additional variant antibodies 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4 8.10.3FG1 or 9.14.4G1 could be made by methods known to one skilled in the art.

Table 1

HUMAN ANTI-M-CSF ANTIBODIES				
MAb	SEQUENCE IDENTIFIER (SEQ ID NOS:)			
	Full Length			
	Heavy		Light	
	DNA	Protein	DNA	Protein
252	1	2	3	4
88	5	6	7	8
100	9	10	11	12
3.8.3		14		16
2.7.3		18		20
1.120.1		22		24
9.14.4I	25	26	27	28
9.14.4	37	38	27	28
9.14.4C-Ser		54		56
9.14.4-CG2		74		56
9.14.4-CG4		78		56
9.14.4-Ser		82	27	28
9.14.4-G1	101	102	27	28
8.10.3F	29	30	31	32
8.10.3	29	30	43	44
8.10.3C-Ser		58		60
8.10.3-CG2		62		60
8.10.3-Ser		90	43	44
8.10.3-CG4		94		60
8.10.3FG1	97	98	31	32
9.7.2IF	33	34	35	36
9.7.2	45	46	47	48
9.7.2C-Ser		50		52
9.7.2-CG2		66		52
9.7.2-CG4		70		52
9.7.2-Ser		86	47	48

Class and Subclass of Anti-M-CSF Antibodies

[0143] The class and subclass of anti-M-CSF antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0144] In some embodiments, the anti-M-CSF antibody is a monoclonal antibody. The anti-M-CSF antibody can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In preferred embodiments, the anti-M-CSF antibody is an IgG and is an IgG1, IgG2, IgG3 or IgG4 subclass. In other preferred embodiments, the antibody is subclass IgG2 or IgG4. In another preferred embodiment, the antibody is subclass IgG1.

Species and Molecular Selectivity

[0145] In another aspect of the invention, the anti-M-CSF antibodies demonstrate both species and molecule selectivity. In some embodiments, the anti-M-CSF antibody binds to human, cynomologus monkey and mouse M-CSF. Following the teachings of the specification, one may determine the species selectivity for the anti-M-CSF antibody using methods well known in the art. For instance, one may determine the species selectivity using Western blot, FACS, ELISA, RIA, a cell proliferation assay, or a M-CSF receptor binding assay. In a preferred embodiment, one may determine the species selectivity using a cell proliferation assay or ELISA.

[0146] In another embodiment, the anti-M-CSF antibody has a selectivity for M-CSF that is at least 100 times greater than its selectivity for GM-/G-CSF. In some embodiments, the anti-M-CSF antibody does not exhibit any appreciable specific

binding to any other protein other than M-CSF. One can determine the selectivity of the anti-M-CSF antibody for M-CSF using methods well known in the art following the teachings of the specification. For instance one can determine the selectivity using Western blot, FACS, ELISA, or RIA.

Identification of M-CSF Epitopes Recognized by Anti- M-CSF Antibodies

[0147] The invention provides a human anti-M-CSF monoclonal antibody that binds to M-CSF and competes with, cross-competes with and/or binds the same epitope and/or binds to M-CSF with the same K_D as (a) an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1; (b) an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102; (c) an antibody that comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; (d) an antibody that comprises both a heavy chain variable region as defined in (b) and a light chain variable region as defined in (c).

[0148] One can determine whether an antibody binds to the same epitope, competes for binding with, cross competes for binding with or has the same K_D an anti-M-CSF antibody by using methods known in the art. In one embodiment, one allows the anti-M-CSF antibody of the invention to bind to M-CSF under saturating conditions and then measures the ability of the test antibody to bind to M-CSF. If the test antibody is able to bind to M-CSF at the same time as the anti-M-CSF antibody, then the test antibody binds to a different epitope as the anti-M-CSF antibody. However, if the test antibody is not able to bind to M-CSF at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the human anti-M-CSF antibody. This experiment can be performed using ELISA, RIA, or FACS. In a preferred embodiment, the experiment is performed using BIACORE™.

Binding Affinity of Anti-M-CSF Antibodies to M-CSF

[0149] In some embodiments of the invention, the anti-M-CSF antibodies bind to M-CSF with high affinity. In some embodiments, the anti-M-CSF antibody binds to M-CSF with a K_D of $1x\ 10^{-7}$ M or less. In other preferred embodiments, the antibody binds to M-CSF with a K_D of $1\ x10^{-8}$ M, $1\ x\ 10^{-9}$ M, $1\ x\ 10^{-10}$ M, $1\ x\ 10^{-11}$ M, $1\ x\ 10^{-12}$ M or less. In certain embodiments, the K_D is 1 pM to 500 pM. In other embodiments, the K_D is between 500 pM to 1 μM. In other embodiments, the K_D is between 1 μM to 100 nM. In other embodiments, the K_D is between 100 mM to 10 nM. In an even more preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a CDR2 of a light chain, and/or a CDR3 of a heavy chain from an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In still another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or that comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. In another preferred embodiment, the antibody binds to M-CSF with substantially the same K_D as an antibody that comprises a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain variable region having an amino acid sequence of the V_L region of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or that comprises a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain variable region having an amino acid sequence of the V_H region of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102.

[0150] In some embodiments, the anti-M-CSF antibody has a low dissociation rate. In some embodiments, the anti-M-CSF antibody has an k_{off} of 2.0×10^{-4} s^{-1} or lower. In other preferred embodiments, the antibody binds to M-CSF with a k_{off} of 2.0×10^{-5} or a k_{off} 2.0×10^{-6} s^{-1} or lower. In some embodiments, the k_{off} is substantially the same as an antibody described herein, such as an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises (a) a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1; or (b) a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain from an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102; or that comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; In another preferred embodiment, the antibody binds to M-CSF with substantially the same k_{off} as an antibody that comprises a CDR2, and may optionally comprise a CDR1 and/or CDR3, of a light chain variable region having an amino acid sequence of SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60; or a CDR3, and may optionally comprise a CDR1 and/or CDR2, of a heavy chain variable region having an amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102.

[0151] The binding affinity and dissociation rate of an anti-M-CSF antibody to a M-CSF can be determined by methods known in the art. The binding affinity can be measured by competitive ELISAs, RIAs, surface plasmon resonance (e.g., by using BIACORE™ technology). The dissociation rate can be measured by surface plasmon resonance. Preferably, the binding affinity and dissociation rate is measured by surface plasmon resonance. More preferably, the binding affinity and dissociation rate are measured using BIACORE™ technology. Example VI exemplifies a method for determining affinity constants of anti-M-CSF monoclonal antibodies by BIACORE™ technology.

Inhibition of M-CSF Activity by Anti-M-CSF Antibody

Inhibition of M-CSF binding to c-fms

[0152] In another embodiment, the invention provides an anti-M-CSF antibody that inhibits the binding of a M-CSF to *c-fms* receptor and blocks or prevents activation of *c-fms*. In an preferred embodiment, the M-CSF is human. In another preferred embodiment, the anti-M-CSF antibody is a human antibody. The IC_{50} can be measured by ELISA, RIA, and cell based assays such as a cell proliferation assay, a whole blood monocyte shape change assay, or a receptor binding inhibition assay. In one embodiment, the antibody or portion thereof inhibits cell proliferation with an IC_{50} of no more than 8.0×10^{-7} M, preferably no more than 3×10^{-7} M, or more preferably no more than 8×10^{-8} M as measured by a cell proliferation assay. In another embodiment, the IC_{50} as measured by a monocyte shape change assay is no more than 2×10^{-6} M, preferably no more than 9.0×10^{-7} M, or more preferably no more than 9×10^{-8} M. In another preferred embodiment, the IC_{50} as measured by a receptor binding assay is no more than 2×10^{-6} M, preferably no more than 8.0×10^{-7} M, or more preferably no more than 7.0×10^{-8} M. Examples III, IV, and V exemplify various types of assays.

[0153] In another aspect anti-M-CSF antibodies of the invention inhibit monocyte/macrophage cell proliferation in response to a M-CSF by at least 20%, more preferably 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 85%, 90%, 95% or 100% compared to the proliferation of cell in the absence of antibody.

Methods of Producing Antibodies and Antibody Producing Cell Lines

Immunization

[0154] In some embodiments, human antibodies are produced by immunizing a non-human animal comprising in its genome some or all of human immunoglobulin heavy chain and light chain loci with a M-CSF antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE™ animal (Abgenix Inc., Fremont, CA). Another non-human animal that may be used is a transgenic mouse produced by Medarex (Medarex, Inc., Princeton, NJ).

[0155] XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green *et al.*, *Nature Genetics* 7:13-21 (1994) and U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00/09560, and WO 00/037504.

[0156] In another aspect, the invention provides a method for making anti-M-CSF antibodies from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human immunoglobulin loci with a M-CSF antigen. One can produce such animals using the methods described in the above-cited documents. The methods disclosed in these documents can be modified as described in U.S. Patent 5,994,619. U.S. Patent 5,994,619 describes methods for producing novel cultural inner cell mass (CICM) cells and cell lines, derived from pigs and cows, and transgenic CICM cells into which heterologous DNA has been inserted. CICM transgenic cells can be used to produce cloned transgenic embryos, fetuses, and offspring. The '619 patent also describes the methods of producing the transgenic animals, that are capable of transmitting the heterologous DNA to their progeny. In preferred embodiments, the non-human animals are rats, sheep, pigs, goats, cattle or horses.

[0157] XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the XENOMOUSE™ mice contain approximately 80% of the

human antibody V gene repertoire through introduction of megabase sized, germline configuration yeast artificial chromosome (YAC) fragments of the human heavy chain loci and kappa light chain loci. In other embodiments, XENOMOUSE™ mice further contain approximately all of the lambda light chain locus. See Mendez *et al.*, *Nature Genetics* 15:146-156 (1997), Green and Jakobovits, *J. Exp. Med.* 188:483-495 (1998), and WO 98/24893, the disclosures of which are hereby incorporated by reference.

[0158] In some embodiments, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus". In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant domain, and a second constant domain (preferably a gamma constant domain) are formed into a construct for insertion into an animal. This approach is described, *inter alia*, in U.S. Patent Nos. 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

[0159] In another aspect, the invention provides a method for making humanized anti-M-CSF antibodies. In some embodiments, non-human animals are immunized with a M-CSF antigen as described below under conditions that permit antibody production. Antibody-producing cells are isolated from the animals, fused with myelomas to produce hybridomas, and nucleic acids encoding the heavy and light chains of an anti-M-CSF antibody of interest are isolated. These nucleic acids are subsequently engineered using techniques known to those of skill in the art and as described further below to reduce the amount of non-human sequence, i.e., to humanize the antibody to reduce the immune response in humans

[0160] In some embodiments, the M-CSF antigen is isolated and/or purified M-CSF. In a preferred embodiment, the M-CSF antigen is human M-CSF. In some embodiments, the M-CSF antigen is a fragment of M-CSF. In some embodiments, the M-CSF fragment is the extracellular domain of M-CSF. In some embodiments, the M-CSF fragment comprises at least one epitope of M-CSF. In other embodiments, the M-CSF antigen is a cell that expresses or overexpresses M-

CSF or an immunogenic fragment thereof on its surface. In some embodiments, the M-CSF antigen is a M-CSF fusion protein. M-CSF can be purified from natural sources using known techniques. Recombinant M-CSF is commercially available.

[0161] Immunization of animals can be by any method known in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane, *supra*, and U.S. Patent 5,994,619. In a preferred embodiment, the M-CSF antigen is administered with an adjuvant to stimulate the immune response. Exemplary adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks. Example I exemplifies a method for producing anti-M-CSF monoclonal antibodies in XENOMOUSE™ mice.

Production of Antibodies and Antibody-Producing Cell Lines

[0162] After immunization of an animal with a M-CSF antigen, antibodies and/or antibody-producing cells can be obtained from the animal. In some embodiments, anti-M-CSF antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-M-CSF antibodies may be purified from the serum.

[0163] In some embodiments, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus, cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or

mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, *supra*. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells are screened using M-CSF, a portion thereof, or a cell expressing M-CSF. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay. An example of ELISA screening is provided in WO 00/37504, incorporated herein by reference.

[0164] Anti-M-CSF antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0165] In a preferred embodiment, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In a more preferred embodiment, the immunized animal is a XENOMOUSE™ animal and the myeloma cell line is a non-secretory mouse myeloma. In an even more preferred embodiment, the myeloma cell line is P3-X63-AG8-653. See, e.g., Example I.

[0166] Thus, in one embodiment, the invention provides methods of producing a cell line that produces a human monoclonal antibody or a fragment thereof directed to M-CSF comprising (a) immunizing a non-human transgenic animal described herein with M-CSF, a portion of M-CSF or a cell or tissue expressing M-CSF; (b) allowing the transgenic animal to mount an immune response to M-CSF; (c) isolating B lymphocytes from a transgenic animal; (d) immortalizing the B lymphocytes; (e) creating individual monoclonal populations of the immortalized B lymphocytes; and (f) screening the immortalized B lymphocytes to identify an antibody directed to M-CSF.

[0167] In another aspect, the invention provides hybridomas that produce an human anti-M-CSF antibody. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In other embodiments, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas.

[0168] In another preferred embodiment, a transgenic animal is immunized with M-CSF, primary cells, e.g., spleen or peripheral blood cells, are isolated from an immunized transgenic animal and individual cells producing antibodies specific for the desired antigen are identified. Polyadenylated mRNA from each individual cell is isolated and reverse transcription polymerase chain reaction (RT-PCR) is performed using sense primers that anneal to variable region sequences, e.g., degenerate primers that recognize most or all of the FR1 regions of human heavy and light chain variable region genes and antisense primers that anneal to constant or joining region sequences. cDNAs of the heavy and light chain variable regions are then cloned and expressed in any suitable host cell, e.g., a myeloma cell, as chimeric antibodies with respective immunoglobulin constant regions, such as the heavy chain and κ or λ constant domains. See Babcook, J.S. *et al.*, *Proc. Natl. Acad. Sci.* USA 93:7843-48, 1996, herein incorporated by reference. Anti M-CSF antibodies may then be identified and isolated as described herein.

[0169] In another embodiment, phage display techniques can be used to provide libraries containing a repertoire of antibodies with varying affinities for M-CSF. For production of such repertoires, it is unnecessary to immortalize the B cells from the immunized animal. Rather, the primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cell, e.g., derived from spleens, is used to prepare an expression library, for example, a phage display library transfected into *E.coli*. The resulting cells are tested for immunoreactivity to M-CSF. Techniques for the identification of high affinity human antibodies from such libraries are described by Griffiths *et al.*, *EMBO J.*, 13:3245-3260 (1994); Nissim *et al.*, *ibid*, pp. 692-698 and by Griffiths *et al.*, *ibid*, 12:725-734. Ultimately, clones from the library are identified which produce binding affinities of a desired magnitude for the antigen and the DNA encoding the product responsible for such binding is recovered and manipulated for standard

recombinant expression. Phage display libraries may also be constructed using previously manipulated nucleotide sequences and screened in a similar fashion. In general, the cDNAs encoding heavy and light chains are independently supplied or linked to form Fv analogs for production in the phage library.

[0170] The phage library is then screened for the antibodies with the highest affinities for M-CSF and the genetic material recovered from the appropriate clone. Further rounds of screening can increase affinity of the original antibody isolated.

[0171] In another aspect, the invention provides hybridomas that produce an human anti-M-CSF antibody. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In other embodiments, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas.

Nucleic Acids, Vectors, Host Cells, and
Recombinant Methods of Making Antibodies

Nucleic Acids

[0172] The present invention also encompasses nucleic acid molecules encoding anti-M-CSF antibodies. In some embodiments, different nucleic acid molecules encode a heavy chain and a light chain of an anti-M-CSF immunoglobulin. In other embodiments, the same nucleic acid molecule encodes a heavy chain an a light chain of an anti-M-CSF immunoglobulin. In one embodiment, the nucleic acid encodes a M-CSF antibody of the invention.

[0173] In some embodiments, the nucleic acid molecule encoding the variable domain of the light chain comprises a human V_κ L5, O12, L2, B3, A27 gene and a Jκ1, Jκ2, Jκ3, or Jκ4 gene.

[0174] In some embodiments, the nucleic acid molecule encoding the light chain, encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations from the germline amino acid sequence. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a V_L amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-conservative amino acid substitutions and/or 1, 2, or 3 non-conservative substitutions compared to germline sequence. Substitutions may be in the CDR regions, the framework regions, or in the constant domain.

[0175] In some embodiments, the nucleic acid molecule encodes a V_L amino acid sequence comprising one or more variants compared to germline sequence that are identical to the variations found in the V_L of one of the antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1.

[0176] In some embodiments, the nucleic acid molecule encodes at least three amino acid mutations compared to the germline sequence found in the V_L of one of the antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4, 8.10.3, or 9.7.2.

[0177] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 252 (SEQ ID NO: 4), 88 (SEQ ID NO: 8), 100 (SEQ ID NO: 12), 3.8.3 (SEQ ID NO: 16), 2.7.3 (SEQ ID NO: 20), 1.120.1 (SEQ ID NO: 24), 9.14.4I (SEQ ID NO: 28), 8.10.3F (SEQ ID NO: 32), 9.7.2IF (SEQ ID NO: 36), 9.14.4 (SEQ ID NO: 28), 8.10.3 (SEQ ID NO: 44), 9.7.2 (SEQ ID NO: 48), 9.7.2C-Ser (SEQ ID NO: 52), 9.14.4C-Ser (SEQ ID NO: 56), 8.10.3C-Ser (SEQ ID NO: 60), 8.10.3-CG2 (SEQ ID NO: 60), 9.7.2-CG2 (SEQ ID NO: 52), 9.7.2-CG4 (SEQ ID NO: 52), 9.14.4-CG2 (SEQ ID NO: 56), 9.14.4-CG4 (SEQ ID NO: 56), 9.14.4-Ser (SEQ ID NO: 28), 9.7.2-Ser (SEQ ID NO: 48), 8.10.3-Ser (SEQ ID NO: 44), 8.10.3-CG4 (SEQ ID NO: 60) 8.10.3FG1 (SEQ ID NO: 32) or 9.14.4G1 (SEQ ID NO: 28), or a portion thereof. In some embodiments, said portion comprises at least the CDR2 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

[0178] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the light chain amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. In some preferred embodiments, the nucleic acid molecule comprises the light chain nucleotide sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47, or a portion thereof.

[0179] In some embodiments, the nucleic acid molecule encodes a V_L amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to a V_L amino acid sequence shown in Figure 1 or to a V_L amino acid

sequences of any one of antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or an amino acid sequence of any one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or that has the light chain nucleic acid sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47.

[0180] In another embodiment, the nucleic acid encodes a full-length light chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or a light chain comprising the amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60 and a constant region of a light chain, or a light chain comprising a mutation. Further, the nucleic acid may comprise the light chain nucleotide sequence of SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47 and the nucleotide sequence encoding a constant region of a light chain, or a nucleic acid molecule encoding a light chain comprise a mutation.

[0181] In another preferred embodiment, the nucleic acid molecule encodes the variable domain of the heavy chain (V_H) that comprises a human V_H 1-18, 3-33, 3-11, 3-23, 3-48, or 3-7 gene sequence or a sequence derived therefrom. In various embodiments, the nucleic acid molecule comprises a human V_H 1-18 gene, a D_H4-23 gene and a human J_H4 gene; a human V_H 3-33 gene, a human D_H1-26 gene and a human J_H4 gene; a human V_H 3-11 gene, a human D_H7-27 gene and a human J_H4 gene; a human V_H 3-11 gene, a human D_H 7-27 gene and a human J_H6 gene; a human V_H 3-23 gene, a human D_H1-26 gene and a human J_H4 gene; a human V_H 3-7 gene, a human D_H6-13 gene and a human J_H4 gene; a human V_H3-11 gene, a human D_H7-27 gene, and a human J_H4b gene; a human V_H3-48 gene, a human

D_H1-26 gene, and a human J_H4b gene; a human V_H3-11 gene, a human D_H6-13 gene, and a human J_H6b gene, or a sequence derived from the human genes.

[0182] In some embodiments, the nucleic acid molecule encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 mutations compared to the germline amino acid sequence of the human V, D or J genes. In some embodiments, said mutations are in the V_H region. In some embodiments, said mutations are in the CDR regions.

[0183] In some embodiments, the nucleic acid molecule encodes one or more amino acid mutations compared to the germline sequence that are identical to amino acid mutations found in the V_H of monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In some embodiments, the nucleic acid encodes at least three amino acid mutations compared to the germline sequences that are identical to at least three amino acid mutations found in one of the above-listed monoclonal antibodies.

[0184] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid sequence of antibody 252 (SEQ ID NO: 2), 88 (SEQ ID NO: 6), 100 (SEQ ID NO: 10), 3.8.3 (SEQ ID NO: 14), 2.7.3 (SEQ ID NO: 18), 1.120.1 (SEQ ID NO: 22), 9.14.41 (SEQ ID NO: 26), 8.10.3F (SEQ ID NO: 30), 9.7.2IF (SEQ ID NO: 34), 9.14.4 (SEQ ID NO: 38), 8.10.3 (SEQ ID NO: 30), 9.7.2 (SEQ ID NO: 46), 9.7.2C-Ser (SEQ ID NO: 50), 9.14.4C-Ser (SEQ ID NO: 54), 8.10.3C-Ser (SEQ ID NO: 58), 8.10.3-CG2 (SEQ ID NO: 62), 9.7.2-CG2 (SEQ ID NO: 66), 9.7.2-CG4 (SEQ ID NO: 70), 9.14.4-CG2 (SEQ ID NO: 74), 9.14.4-CG4 (SEQ ID NO: 78), 9.14.4-Ser (SEQ ID NO: 82), 9.7.2-Ser (SEQ ID NO: 86), 8.10.3-Ser (SEQ ID NO: 90), 8.10.3-CG4 (SEQ ID NO: 94), 8.10.3FG1 (SEQ ID NO: 98) or 9.14.4-G1 (SEQ ID NO: 102), or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

[0185] In some embodiments, the nucleic acid molecule comprises a heavy chain nucleotide sequence that encodes the amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102. In some preferred embodiments, the nucleic acid molecule comprises at least a portion of the heavy chain nucleotide sequence of SEQ ID NO: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In some embodiments, said portion encodes the V_H region, a CDR3 region, all three CDR regions, or a contiguous region including CDR1-CDR3.

[0186] In some embodiments, the nucleic acid molecule encodes a V_H amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the V_H amino acid sequences shown in Figure 4 or to a V_H amino acid sequence of any one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the heavy chain amino acid sequence of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102 or that has the nucleotide sequence of SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101.

[0187] In another embodiment, the nucleic acid encodes a full-length heavy chain of an antibody selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or a heavy chain having the amino acid sequence of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102 and a constant region of a heavy chain, or a heavy chain comprising a mutation. Further, the nucleic acid may comprise the heavy chain nucleotide sequence of SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101 and a nucleotide sequence encoding a constant region of a light chain, or a nucleic acid molecule encoding a heavy chain comprising a mutation.

[0188] A nucleic acid molecule encoding the heavy or entire light chain of an anti-M-CSF antibody or portions thereof can be isolated from any source that

produces such antibody. In various embodiments, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with M-CSF or from an immortalized cell derived from such a B cell that expresses an anti-M-CSF antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g., Sambrook *et al.* The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human immunoglobulin-producing cell from a non-human transgenic animal. In an even more preferred embodiment, the human immunoglobulin producing cell is isolated from a XENOMOUSE™ animal. In another embodiment, the human immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described above. In another embodiment, the nucleic acid is isolated from a non-human, non-transgenic animal. The nucleic acid molecules isolated from a non-human, non-transgenic animal may be used, e.g., for humanized antibodies.

[0189] In some embodiments, a nucleic acid encoding a heavy chain of an anti-M-CSF antibody of the invention can comprise a nucleotide sequence encoding a V_H domain of the invention joined in-frame to a nucleotide sequence encoding a heavy chain constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-M-CSF antibody of the invention can comprise a nucleotide sequence encoding a V_L domain of the invention joined in-frame to a nucleotide sequence encoding a light chain constant domain from any source.

[0190] In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (V_H) and light (V_L) chains are "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V_H or V_L domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (C_H) or light chain (C_L) constant domains, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector, and the V_L segment is operatively linked to the C_L segment within the vector. In another embodiment, nucleic acid molecules encoding the V_H and/or V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains

to a nucleic acid molecule encoding a C_H and/or C_L domain using standard molecular biological techniques. Nucleic acid sequences of human heavy and light chain immunoglobulin constant domain genes are known in the art. See, e.g., Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed., NIH Publ. No. 91-3242, 1991. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-M-CSF antibody isolated.

[0191] The nucleic acid molecules may be used to recombinantly express large quantities of anti-M-CSF antibodies. The nucleic acid molecules also may be used to produce chimeric antibodies, bispecific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described further below. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for antibody humanization, also as described below.

[0192] In another embodiment, a nucleic acid molecule of the invention is used as a probe or PCR primer for a specific antibody sequence. For instance, the nucleic acid can be used as a probe in diagnostic methods or as a PCR primer to amplify regions of DNA that could be used, inter alia, to isolate additional nucleic acid molecules encoding variable domains of anti-M-CSF antibodies. In some embodiments, the nucleic acid molecules are oligonucleotides. In some embodiments, the oligonucleotides are from highly variable regions of the heavy and light chains of the antibody of interest. In some embodiments, the oligonucleotides encode all or a part of one or more of the CDRs of antibody 252, 88, 100, 3.8.3, 2.7.3, or 1.120.1, or variants thereof described herein.

Vectors

[0193] The invention provides vectors comprising nucleic acid molecules that encode the heavy chain of an anti-M-CSF antibody of the invention or an antigen-binding portion thereof. The invention also provides vectors comprising nucleic acid molecules that encode the light chain of such antibodies or antigen-binding portion thereof. The invention further provides vectors comprising nucleic acid

molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[0194] In some embodiments, the anti-M-CSF antibodies, or antigen-binding portions of the invention are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and transnational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and transnational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0195] A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0196] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Patent No. 5,168,062, U.S. Patent No. 4,510,245 and U.S. Patent No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, e.g., United States Patents 6,517,529, herein incorporated by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

[0197] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neomycin resistance gene (for G418 selection), and the glutamate synthetase gene.

Non-Hybridoma Host Cells and Methods of Recombinantly Producing Protein

[0198] Nucleic acid molecules encoding anti-M-CSF antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, e.g., U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). Methods of transforming plant cells are well known in the art, including, e.g., Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

[0199] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Plant host cells include, e.g., Nicotiana, Arabidopsis, duckweed, corn, wheat, potato, etc. Bacterial host cells include

E. coli and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[0200] Further, expression of antibodies of the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

[0201] It is possible that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation from each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation state or pattern or modification of the antibodies.

Transgenic Animals and Plants

[0202] Anti-M-CSF antibodies of the invention also can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, anti-M-CSF antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957. In some embodiments, non-human transgenic animals that comprise human immunoglobulin loci are immunized with M-CSF or an immunogenic portion thereof, as described above. Methods for making antibodies in plants, yeast or fungi/algae are described, e.g., in US patents 6,046,037 and US 5,959,177.

[0203] In some embodiments, non-human transgenic animals or plants are produced by introducing one or more nucleic acid molecules encoding an anti-M-CSF antibody of the invention into the animal or plant by standard transgenic techniques. See Hogan and United States Patent 6,417,429, *supra*. The transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic

cells. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. See, e.g., Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual* 2ed., Cold Spring Harbor Press (1999); Jackson *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press (2000); and Pinkert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press (1999). In some embodiments, the transgenic non-human animals have a targeted disruption and replacement by a targeting construct that encodes a heavy chain and/or a light chain of interest. In a preferred embodiment, the transgenic animals comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to M-CSF, preferably human M-CSF. In some embodiments, the transgenic animals comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-M-CSF antibodies may be made in any transgenic animal. In a preferred embodiment, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses. The non-human transgenic animal expresses said encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

Phage Display Libraries

[0204] The invention provides a method for producing an anti-M-CSF antibody or antigen-binding portion thereof comprising the steps of synthesizing a library of human antibodies on phage, screening the library with M-CSF or a portion thereof, isolating phage that bind M-CSF, and obtaining the antibody from the phage. By way of example, one method for preparing the library of antibodies for use in phage display techniques comprises the steps of immunizing a non-human animal comprising human immunoglobulin loci with M-CSF or an antigenic portion thereof to create an immune response, extracting antibody producing cells from the immunized animal; isolating RNA from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using a primer, and inserting the cDNA into a phage display vector such that antibodies are expressed on the phage. Recombinant anti-M-CSF antibodies of the invention may be obtained in this way.

[0205] Recombinant anti-M-CSF human antibodies of the invention can be isolated by screening a recombinant combinatorial antibody library. Preferably the library is a scFv phage display library, generated using human V_L and V_H cDNAs prepared from mRNA isolated from B cells. Methodologies for preparing and screening such libraries are known in the art. There are commercially available kits for generating phage display libraries (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; Fuchs *et al.*, *Bio/Technology* 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas* 3:81-85 (1992); Huse *et al.*, *Science* 246:1275-1281 (1989); McCafferty *et al.*, *Nature* 348:552-554 (1990); Griffiths *et al.*, *EMBO J.* 12:725-734 (1993); Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992); Clackson *et al.*, *Nature* 352:624-628 (1991); Gram *et al.*, *Proc. Natl. Acad. Sci. USA* 89:3576-3580 (1992); Garrad *et al.*, *Bio/Technology* 9:1373-1377 (1991); Hoogenboom *et al.*, *Nuc. Acid Res.* 19:4133-4137 (1991); and Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991).

[0206] In one embodiment, to isolate a human anti-M-CSF antibodies with the desired characteristics, a human anti-M-CSF antibody as described herein is first used to select human heavy and light chain sequences having similar binding activity toward M-CSF, using the epitope imprinting methods described in PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* 348:552-554 (1990); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993). The scFv antibody libraries preferably are screened using human M-CSF as the antigen.

[0207] Once initial human V_L and V_H domains are selected, "mix and match" experiments are performed, in which different pairs of the initially selected V_L and V_H segments are screened for M-CSF binding to select preferred V_L/V_H pair combinations. Additionally, to further improve the quality of the antibody, the V_L

and V_H segments of the preferred V_L/V_H pair(s) can be randomly mutated, preferably within the CDR3 region of V_H and/or V_L, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying V_H and V_L domains using PCR primers complimentary to the V_H CDR3 or V_L CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be re-screened for binding to M-CSF.

[0208] Following screening and isolation of an anti-M-CSF antibody of the invention from a recombinant immunoglobulin display library, nucleic acids encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can further be manipulated to create other antibody forms of the invention, as described below. To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described above.

Class switching

[0209] Another aspect of the invention provides a method for converting the class or subclass of an anti-M-CSF antibody to another class or subclass. In some embodiments, a nucleic acid molecule encoding a V_L or V_H that does not include any nucleic acid sequences encoding C_L or C_H is isolated using methods well-known in the art. The nucleic acid molecule then is operatively linked to a nucleic acid sequence encoding a C_L or C_H from a desired immunoglobulin class or subclass. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as described above. For example, an anti-M-CSF antibody that was originally IgM can be class switched to an IgG. Further, the class switching may be used to convert one IgG subclass to another, e.g., from

IgG1 to IgG2. Another method for producing an antibody of the invention comprising a desired isotype comprises the steps of isolating a nucleic acid encoding a heavy chain of an anti-M-CSF antibody and a nucleic acid encoding a light chain of an anti-M-CSF antibody, isolating the sequence encoding the V_H region, ligating the V_H sequence to a sequence encoding a heavy chain constant domain of the desired isotype, expressing the light chain gene and the heavy chain construct in a cell, and collecting the anti-M-CSF antibody with the desired isotype.

[0210] In some embodiments, anti-M-CSF antibodies of the invention have the serine at position 228 (according to the EU-numbering convention) of the heavy chain changed to a proline. Accordingly, the CPSC sub-sequence in the F_C region of IgG4 becomes CPPC, which is the sub-sequence in IgG1. (Aalberse, R.C. and Schuurman, J., *Immunology*, 105:9-19 (2002)). For example, the serine at residue 243 SEQ ID NO: 46 (which corresponds to reside 228 in the EU-numbering convention) would become proline. Similarly, the serine at residue 242 of SEQ ID NO: 38 (which corresponds to reside 228 in the EU-numbering convention) would become proline. In some embodiments, the framework region of the IgG4 antibody can be back-mutated to the germline framework sequence. Some embodiments comprise both the back-mutates framework region and the serine to proline change in the F_C region. See, e.g., SEQ ID NO: 54 (antibody 9.14.4C-Ser) and SEQ ID NO: 58 (antibody 8.10.3C-Ser) in Table 1.

Deimmunized Antibodies

[0211] Another way of producing antibodies with reduced immunogenicity is the deimmunization of antibodies. In another aspect of the invention, the antibody may be deimmunized using the techniques described in, e.g., PCT Publication Nos. WO98/52976 and WO00/34317 (which incorporated herein by reference in their entirety).

Mutated Antibodies

[0212] In another embodiment, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-M-CSF antibodies. The antibodies may be

mutated in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the K_D of the antibody for M-CSF, to increase or decrease k_{off}, or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook *et al.* and Ausubel *et al.*, *supra*. In a preferred embodiment, mutations are made at an amino acid residue that is known to be changed compared to germline in a variable domain of an anti-M-CSF antibody. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain, or in a constant domain of a monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of a heavy chain amino acid sequence selected from SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98 or 102, or whose heavy chain nucleotide sequence is presented in SEQ ID NOS: 1, 5, 9, 25, 29, 33, 37, 45, 97 or 101. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of a light chain amino acid sequence selected from SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, 48, 52, 56 or 60, or whose light chain nucleotide sequence is presented in SEQ ID NOS: 3, 7, 11, 27, 31, 35, 43 or 47.

[0213] In one embodiment, the framework region is mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-M-CSF antibody. See, e.g., PCT Publication No. WO 00/09560, herein incorporated by reference. A mutation in a framework region or constant domain also can be made to alter the immunogenicity of the

antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity (ADCC). According to the invention, a single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant domain.

[0214] In some embodiments, there are from 1 to 8 including any number in between, amino acid mutations in either the V_H or V_L domains of the mutated anti-M-CSF antibody compared to the anti-M-CSF antibody prior to mutation. In any of the above, the mutations may occur in one or more CDR regions. Further, any of the mutations can be conservative amino acid substitutions. In some embodiments, there are no more than 5, 4, 3, 2, or 1 amino acid changes in the constant domains.

Modified Antibodies

[0215] In another embodiment, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-M-CSF antibody of the invention linked to another polypeptide. In a preferred embodiment, only the variable domains of the anti-M-CSF antibody are linked to the polypeptide. In another preferred embodiment, the V_H domain of an anti-M-CSF antibody is linked to a first polypeptide, while the V_L domain of an anti-M-CSF antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V_H and V_L domains can interact with one another to form an antibody binding site. In another preferred embodiment, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (see below under Single Chain Antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. The fusion antibody is useful for directing a polypeptide to a M-CSF-expressing cell or tissue. The polypeptide may be a therapeutic agent, such as a toxin, growth factor or other regulatory protein, or may be a diagnostic agent, such as an enzyme that may be easily visualized, such as horseradish peroxidase. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one

wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

[0216] To create a single chain antibody, (scFv) the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence $(Gly_4$ -$Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker. See, e.g., Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); McCafferty *et al.*, *Nature* 348:552-554 (1990). The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to M-CSF and to another molecule.

[0217] In other embodiments, other modified antibodies may be prepared using anti-M-CSF antibody-encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill *et al.*, *Protein Eng.* 10: 949-57 (1997)), "Minibodies" (Martin *et al.*, *EMBO J.* 13: 5303-9 (1994)), "Diabodies" (Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)), or "Janusins" (Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991) and Traunecker *et al.*, *Int. J. Cancer* (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the specification.

[0218] Bispecific antibodies or antigen-binding fragments can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny *et al.*, *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" or "Janusins." In some embodiments, the bispecific antibody binds to two different epitopes of M-CSF. In some embodiments, the bispecific antibody has a first heavy chain and a first light chain from monoclonal antibody 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, or 9.7.2 and an additional antibody heavy chain and light chain. In some embodiments, the additional light chain and heavy chain also are

from one of the above-identified monoclonal antibodies, but are different from the first heavy and light chains.

[0219] In some embodiments, the modified antibodies described above are prepared using one or more of the variable domains or CDR regions from a human anti-M-CSF monoclonal antibody provided herein, from an amino acid sequence of said monoclonal antibody, or from a heavy chain or light chain encoded by a nucleic acid sequence encoding said monoclonal antibody.

Derivatized and Labeled Antibodies

[0220] An anti-M-CSF antibody or antigen-binding portion of the invention can be derivatized or linked to another molecule (e.g., another peptide or protein). In general, the antibodies or portion thereof is derivatized such that the M-CSF binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies and antibody portions of the invention are intended to include both intact and modified forms of the human anti-M-CSF antibodies described herein. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detection agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0221] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

[0222] Another type of derivatized antibody is a labeled antibody. Useful detection agents with which an antibody or antigen-binding portion of the invention may be derivatized include fluorescent compounds, including

fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. An antibody can also be labeled with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody can also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody can also be labeled with a predetermined polypeptide epitope recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0223] An anti-M-CSF antibody can also be labeled with a radiolabeled amino acid. The radiolabeled anti-M-CSF antibody can be used for both diagnostic and therapeutic purposes. For instance, the radiolabeled anti-M-CSF antibody can be used to detect M-CSF-expressing tumors by x-ray or other diagnostic techniques. Further, the radiolabeled anti-M-CSF antibody can be used therapeutically as a toxin for cancerous cells or tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides – ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, and ^{131}I.

[0224] An anti-M-CSF antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups are useful to improve the biological characteristics of the antibody, e.g., to increase serum half-life or to increase tissue binding.

Pharmaceutical Compositions and Kits

[0225] The invention also relates to compositions comprising a human anti-M-CSF antagonist antibody for the treatment of subjects in need of treatment for rheumatoid arthritis, osteoporosis, or atherosclerosis. In some embodiments, the subject of treatment is a human. In other embodiments, the subject is a veterinary

subject. Hyperproliferative disorders where monocytes play a role that may be treated by an antagonist anti-M-CSF antibody of the invention can involve any tissue or organ and include but are not limited to brain, lung, squamous cell, bladder, gastric, pancreatic, breast, head, neck, liver, renal, ovarian, prostate, colorectal, esophageal, gynecological, nasopharynx, or thyroid cancers, melanomas, lymphomas, leukemias or multiple myelomas. In particular, human antagonist anti-M-CSF antibodies of the invention are useful to treat or prevent carcinomas of the breast, prostate, colon and lung.

[0226] This invention also encompasses compositions for the treatment of a condition selected from the group consisting of arthritis, psoriatic arthritis, Reiter's syndrome, gout, traumatic arthritis, rubella arthritis and acute synovitis, rheumatoid arthritis, rheumatoid spondylitis, ankylosing spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, Alzheimer's disease, stroke, neurotrauma, asthma, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption disease, osteoporosis, restenosis, cardiac and renal reperfusion injury, thrombosis, glomerularonephritis, diabetes, graft vs. host reaction, allograft rejection, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, muscle degeneration, eczema, contact dermatitis, psoriasis, sunburn, or conjunctivitis shock in a mammal, including a human, comprising an amount of a human anti-M-CSF monoclonal antibody of the invention effective in such treatment and a pharmaceutically acceptable carrier.

[0227] Treatment may involve administration of one or more antagonist anti-M-CSF monoclonal antibodies of the invention, or antigen-binding fragments thereof, alone or with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable carriers are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars,

polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

[0228] Anti-M-CSF antibodies of the invention and compositions comprising them, can be administered in combination with one or more other therapeutic, diagnostic or prophylactic agents. Additional therapeutic agents include other anti-neoplastic, anti-tumor, anti-angiogenic or chemotherapeutic agents. Such additional agents may be included in the same composition or administered separately. In some embodiments, one or more inhibitory anti-M-CSF antibodies of the invention can be used as a vaccine or as adjuvants to a vaccine.

[0229] The compositions of this invention may be in a variety of forms, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection. In another embodiment, the invention includes a method of treating a subject in need thereof with an antibody or an antigen-binding portion thereof that specifically binds to M-CSF comprising the steps of : (a) administering an effective amount of an isolated nucleic acid molecule encoding the heavy chain or the antigen-binding portion thereof, an isolated nucleic acid molecule encoding the light chain or the antigen-binding portion thereof, or both the nucleic acid molecules encoding the light chain and the heavy chain or antigen-binding portions thereof; and (b) expressing the nucleic acid molecule.

[0230] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-M-CSF antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0231] The antibodies of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, or intravenous infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

[0232] In certain embodiments, the antibody compositions active compound may be prepared with a carrier that will protect the antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems* (J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978).

[0233] In certain embodiments, an anti-M-CSF antibody of the invention can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the anti-M-CSF antibodies can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[0234] Additional active compounds also can be incorporated into the compositions. In certain embodiments, an anti-M-CSF antibody of the invention is co-formulated with and/or co-administered with one or more additional therapeutic agents. These agents include antibodies that bind other targets, antineoplastic agents, antitumor agents, chemotherapeutic agents, peptide analogues that inhibit M-CSF, soluble *c-fms* that can bind M-CSF, one or more chemical agents that inhibit M-CSF, anti-inflammatory agents, anti-coagulants, agents that lower blood pressure (i.e, angiotensin-converting enzyme (ACE) inhibitors). Such combination therapies may require lower dosages of the anti-M-CSF antibody as well as the co-administered agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0235] Inhibitory anti-M-CSF antibodies of the invention and compositions comprising them also may be administered in combination with other therapeutic regimens, in particular in combination with radiation treatment for cancer. The compounds of the present invention may also be used in combination with anticancer agents such as endostatin and angiostatin or cytotoxic drugs such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and alkaloids, such as vincristine, farnesyl transferase inhibitors, VEGF inhibitors, and antimetabolites such as methotrexate.

[0236] The compounds of the invention may also be used in combination with antiviral agents such as Viracept, AZT, aciclovir and famciclovir, and antisepsis compounds such as Valant.

[0237] The compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antigen-binding portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0238] Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the anti-M-CSF antibody or portion and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

[0239] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the

invention is 0.025 to 50 mg/kg, more preferably 0.1 to 50 mg/kg, more preferably 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0240] Another aspect of the present invention provides kits comprising an anti-M-CSF antibody or antigen-binding portion of the invention or a composition comprising such an antibody or portion. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit also can include instructions for use in a diagnostic or therapeutic method. In a preferred embodiment, the kit includes the antibody or a composition comprising it and a diagnostic agent that can be used in a method described below. In another preferred embodiment, the kit includes the antibody or a composition comprising it and one or more therapeutic agents that can be used in a method described below. One embodiment of the invention is a kit comprising a container, instructions on the administration of an anti-M-CSF antibody to a human suffering from an inflammatory disease, or instructions for measuring the number of CD14+CD16+ monocytes in a biological sample and an anti-M-CSF antibody.

[0241] This invention also relates to compositions for inhibiting abnormal cell growth in a mammal comprising an amount of an antibody of the invention in combination with an amount of a chemotherapeutic agent, wherein the amounts of the compound, salt, solvate, or prodrug, and of the chemotherapeutic agent are together effective in inhibiting abnormal cell growth. Many chemotherapeutic agents are known in the art. In some embodiments, the chemotherapeutic agent is selected from the group consisting of mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, e.g. anti-androgens, and anti-angiogenesis agents.

[0242] Anti-angiogenic agents, such as MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with an anti-M-CSF antibody of the invention. Examples of useful COX-II inhibitors include CELEBREX™ (celecoxib), valdecoxib, and rofecoxib. Examples of useful matrix metalloproteinase inhibitors are described in WO 96/33172 (published October 24, 1996), WO 96/27583 (published March 7, 1996), European Patent Application No. 97304971.1 (filed July 8, 1997), European Patent Application No. 99308617.2 (filed October 29, 1999), WO 98/07697 (published February 26, 1998), WO 98/03516 (published January 29, 1998), WO 98/34918 (published August 13, 1998), WO 98/34915 (published August 13, 1998), WO 98/33768 (published August 6, 1998), WO 98/30566 (published July 16, 1998), European Patent Publication 606,046 (published July 13, 1994), European Patent Publication 931,788 (published July 28, 1999), WO 90/05719 (published May 31, 1990), WO 99/52910 (published October 21, 1999), WO 99/52889 (published October 21, 1999), WO 99/29667 (published June 17, 1999), PCT International Application No. PCT/IB98/01113 (filed July 21, 1998), European Patent Application No. 99302232.1 (filed March 25, 1999), Great Britain patent application number 9912961.1 (filed June 3, 1999), U.S. Provisional Application No. 60/148,464 (filed August 12, 1999), U.S. Patent 5,863,949 (issued January 26, 1999), U.S. Patent 5,861,510 (issued January 19, 1999), and European Patent Publication 780,386 (published June 25, 1997), all of which are incorporated herein in their entireties by reference. Preferred MMP inhibitors are those that do not demonstrate arthralgia. More preferred, are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e. MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13). Some specific examples of MMP inhibitors useful in the present invention are AG-3340, RO 32-3555, RS 13-0830, and the compounds recited in the following list: 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclopentyl)-amino]-propionic acid; 3-exo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; (2R, 3R) 1-[4-(2-chloro-4-fluoro-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-

carboxylic acid hydroxyamide; 4-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclobutyl)-amino]-propionic acid; 4-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide; (R) 3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-3-carboxylic acid hydroxyamide; (2R, 3R) 1-[4-(4-fluoro-2-methyl-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-1-methyl-ethyl)-amino]-propionic acid; 3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(4-hydroxycarbamoyl-tetrahydro-pyran-4-yl)-amino]-propionic acid; 3-exo-3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; 3-endo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; and (R) 3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-furan-3-carboxylic acid hydroxyamide; and pharmaceutically acceptable salts and solvates of said compounds.

[0243] A compound comprising a human anti-M-CSF monoclonal antibody of the invention can also be used with signal transduction inhibitors, such as agents that can inhibit EGF-R (epidermal growth factor receptor) responses, such as EGF-R antibodies, EGF antibodies, and molecules that are EGF-R inhibitors; VEGF (vascular endothelial growth factor) inhibitors, such as VEGF receptors and molecules that can inhibit VEGF; and erbB2 receptor inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor, for example, HERCEPTIN™ (Genentech, Inc.). EGF-R inhibitors are described in, for example in WO 95/19970 (published July 27, 1995), WO 98/14451 (published April 9, 1998), WO 98/02434 (published January 22, 1998), and United States Patent 5,747,498 (issued May 5, 1998), and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225 and anti-EGFR 22Mab (ImClone Systems Incorporated), ABX-EGF (Abgenix/Cell Genesys), EMD-7200 (Merck KgaA), EMD-5590 (Merck KgaA), MDX-447/H-477 (Medarex Inc. and Merck KgaA), and the compounds ZD-1834, ZD-1838 and ZD-1839 (AstraZeneca), PKI-166

(Novartis), PKI-166/CGP-75166 (Novartis), PTK 787 (Novartis), CP 701 (Cephalon), leflunomide (Pharmacia/Sugen), CI-1033 (Warner Lambert Parke Davis), CI-1033/PD 183,805 (Warner Lambert Parke Davis), CL-387,785 (Wyeth-Ayerst), BBR-1611 (Boehringer Mannheim GmbH/Roche), Naamidine A (Bristol Myers Squibb), RC-3940-II (Pharmacia), BIBX-1382 (Boehringer Ingelheim), OLX-103 (Merck & Co.), VRCTC-310 (Ventech Research), EGF fusion toxin (Seragen Inc.), DAB-389 (Seragen/Lilgand), ZM-252808 (Imperial Cancer Research Fund), RG-50864 (INSERM), LFM-A12 (Parker Hughes Cancer Center), WHI-P97 (Parker Hughes Cancer Center), GW-282974 (Glaxo), KT-8391 (Kyowa Hakko) and EGF-R Vaccine (York Medical/Centro de Immunologia Molecular (CIM)). These and other EGF-R-inhibiting agents can be used in the present invention.

[0244] VEGF inhibitors, for example SU-5416 and SU-6668 (Sugen Inc.), AVASTIN™ (Genentech), SH-268 (Schering), and NX-1838 (NeXstar) can also be combined with the compound of the present invention. VEGF inhibitors are described in, for example in WO 99/24440 (published May 20, 1999), PCT International Application PCT/IB99/00797 (filed May 3, 1999), in WO 95/21613 (published August 17, 1995), WO 99/61422 (published December 2, 1999), United States Patent 5,834,504 (issued November 10, 1998), WO 98/50356 (published November 12, 1998), United States Patent 5,883,113 (issued March 16, 1999), United States Patent 5,886,020 (issued March 23, 1999), United States Patent 5,792,783 (issued August 11, 1998), WO 99/10349 (published March 4, 1999), WO 97/32856 (published September 12, 1997), WO 97/22596 (published June 26, 1997), WO 98/54093 (published December 3, 1998), WO 98/02438 (published January 22, 1998), WO 99/16755 (published April 8, 1999), and WO 98/02437 (published January 22, 1998), all of which are incorporated herein in their entireties by reference. Other examples of some specific VEGF inhibitors useful in the present invention are IM862 (Cytran Inc.); anti-VEGF monoclonal antibody of Genentech, Inc.; and angiozyme, a synthetic ribozyme from Ribozyme and Chiron. These and other VEGF inhibitors can be used in the present invention as described herein. ErbB2 receptor inhibitors, such as GW-282974 (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc.) and 2B-1

(Chiron), can furthermore be combined with the compound of the invention, for example those indicated in WO 98/02434 (published January 22, 1998), WO 99/35146 (published July 15, 1999), WO 99/35132 (published July 15, 1999), WO 98/02437 (published January 22, 1998), WO 97/13760 (published April 17, 1997), WO 95/19970 (published July 27, 1995), United States Patent 5,587,458 (issued December 24, 1996), and United States Patent 5,877,305 (issued March 2, 1999), which are all hereby incorporated herein in their entireties by reference. ErbB2 receptor inhibitors useful in the present invention are also described in United States Patent 6,465,449 (issued October 15, 2002), and in United States Patent 6,284,764 (issued September 4, 2001), both of which are incorporated in their entireties herein by reference. The erbB2 receptor inhibitor compounds and substance described in the aforementioned PCT applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the compound of the present invention in accordance with the present invention.

[0245] Anti-survival agents include anti-IGF-IR antibodies and anti-integrin agents, such as anti-integrin antibodies.

[0246] Anti-inflammatory agents can be used in conjunction with an anti-M-CSF antibody of the invention. For the treatment of rheumatoid arthritis, the human anti-M-CSF antibodies of the invention may be combined with agents such as TNF-α inhibitors such as TNF drugs (such as REMICADE™, CDP-870 and HUMIRA™) and TNF receptor immunoglobulin molecules (such as ENBREL™), IL-1 inhibitors, receptor antagonists or soluble IL-1ra (e.g. Kineret or ICE inhibitors), COX-2 inhibitors (such as celecoxib , rofecoxib, valdecoxib and etoricoxib), metalloprotease inhibitors (preferably MMP-13 selective inhibitors), p2X7 inhibitors, α2δ ligands (such as NEUROTIN™ AND PREGABALIN™), low dose methotrexate, leflunomide, hydroxychloroquine, d-penicillamine, auranofin or parenteral or oral gold. The compounds of the invention can also be used in combination with existing therapeutic agents for the treatment of osteoarthritis. Suitable agents to be used in combination include standard non-steroidal anti-inflammatory agents (hereinafter NSAID's) such as piroxicam, diclofenac, propionic acids such as naproxen, flurbiprofen, fenoprofen, ketoprofen

and ibuprofen, fenamates such as mefenamic acid, indomethacin, sulindac, apazone, pyrazolones such as phenylbutazone, salicylates such as aspirin, COX-2 inhibitors such as celecoxib, valdecoxib, rofecoxib and etoricoxib, analgesics and intraarticular therapies such as corticosteroids and hyaluronic acids such as hyalgan and synvisc.

[0247] Anti-coagulant agents can be used in conjunction with an anti-M-CSF antibody of the invention. Examples of anti-coagulant agents include, but are not limited to, warfarin (COUMADIN™), heparin, and enoxaparin (LOVENOX™).

[0248] The human anti-M-CSF antibodies of the present invention may also be used in combination with cardiovascular agents such as calcium channel blockers, lipid lowering agents such as statins, fibrates, beta-blockers, Ace inhibitors, Angiotensin-2 receptor antagonists and platelet aggregation inhibitors. The compounds of the present invention may also be used in combination with CNS agents such as antidepressants (such as sertraline), anti-Parkinsonian drugs (such as deprenyl, L-dopa, REQUIP™, MIRAPEX™, MAOB inhibitors such as selegine and rasagiline, comP inhibitors such as Tasmar, A-2 inhibitors, dopamine reuptake inhibitors, NMDA antagonists, Nicotine agonists, Dopamine agonists and inhibitors of neuronal nitric oxide synthase), and anti-Alzheimer's drugs such as donepezil, tacrine, α2δ LIGANDS (such NEUROTIN™ and PREGABALIN™) inhibitors, COX-2 inhibitors, propentofylline or metryfonate.

[0249] The human anti-M-CSF antibodies of the present invention may also be used in combination with osteoporosis agents such as roloxifene, droloxifene, lasofoxifene or fosomax and immunosuppressant agents such as FK-506 and rapamycin.

Diagnostic Methods of Use

[0250] In another aspect, the invention provides diagnostic methods. The anti-M-CSF antibodies can be used to detect M-CSF in a biological sample *in vitro* or *in vivo*. In one embodiment, the invention provides a method for diagnosing the presence or location of a M-CSF-expressing tumor in a subject in need thereof, comprising the steps of injecting the antibody into the subject, determining the expression of M-CSF in the subject by localizing where the antibody has bound,

comparing the expression in the subject with that of a normal reference subject or standard, and diagnosing the presence or location of the tumor.

[0251] The anti-M-CSF antibodies can be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, FACS, tissue immunohistochemistry, Western blot or immunoprecipitation. The anti-M-CSF antibodies of the invention can be used to detect M-CSF from humans. In another embodiment, the anti-M-CSF antibodies can be used to detect M-CSF from primates such as cynomologus monkey, rhesus monkeys, chimpanzees or apes. The invention provides a method for detecting M-CSF in a biological sample comprising contacting a biological sample with an anti-M-CSF antibody of the invention and detecting the bound antibody. In one embodiment, the anti-M-CSF antibody is directly labeled with a detectable label. In another embodiment, the anti-M-CSF antibody (the first antibody) is unlabeled and a second antibody or other molecule that can bind the anti-M-CSF antibody is labeled. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the particular species and class of the first antibody. For example, if the anti-M-CSF antibody is a human IgG, then the secondary antibody could be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially, e.g., from Pierce Chemical Co.

[0252] Suitable labels for the antibody or secondary antibody have been disclosed *supra*, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[0253] In other embodiments, M-CSF can be assayed in a biological sample by a competition immunoassay utilizing M-CSF standards labeled with a detectable

substance and an unlabeled anti-M-CSF antibody. In this assay, the biological sample, the labeled M-CSF standards and the anti-M-CSF antibody are combined and the amount of labeled M-CSF standard bound to the unlabeled antibody is determined. The amount of M-CSF in the biological sample is inversely proportional to the amount of labeled M-CSF standard bound to the anti-M-CSF antibody.

[0254] One can use the immunoassays disclosed above for a number of purposes. For example, the anti-M-CSF antibodies can be used to detect M-CSF in cells or on the surface of cells in cell culture, or secreted into the tissue culture medium. The anti-M-CSF antibodies can be used to determine the amount of M-CSF on the surface of cells or secreted into the tissue culture medium that have been treated with various compounds. This method can be used to identify compounds that are useful to inhibit or activate M-CSF expression or secretion. According to this method, one sample of cells is treated with a test compound for a period of time while another sample is left untreated. If the total level of M-CSF is to be measured, the cells are lysed and the total M-CSF level is measured using one of the immunoassays described above. The total level of M-CSF in the treated versus the untreated cells is compared to determine the effect of the test compound.

[0255] An immunoassay for measuring total M-CSF levels is an ELISA or Western blot. If the cell surface level of M-CSF is to be measured, the cells are not lysed, and the M-CSF cell surface levels can be measured using one of the immunoassays described above. An immunoassay for determining cell surface levels of M-CSF can include the steps of labeling the cell surface proteins with a detectable label, such as biotin or ^{125}I, immunoprecipitating the M-CSF with an anti-M-CSF antibody and then detecting the labeled M-CSF. Another immunoassay for determining the localization of M-CSF, e.g., cell surface levels, can be immunohistochemistry. Methods such as ELISA, RIA, Western blot, immunohistochemistry, cell surface labeling of integral membrane proteins and immunoprecipitation are well known in the art. See, e.g., Harlow and Lane, *supra*. In addition, the immunoassays can be scaled up for high throughput screening in order to test a large number of compounds for inhibition or activation of M-CSF.

[0256] Another example of an immunoassay for measuring secreted M-CSF levels can be an antigen capture assay, ELISA, immunohistochemistry assay, Western blot and the like using antibodies of the invention. If secreted M-CSF is to be measured, cell culture media or body fluid, such as blood serum, urine, or synovial fluid, can be assayed for secreted M-CSF and/or cells can be lysed to release produced, but not yet secreted M-CSF. An immunoassay for determining secreted levels of M-CSF includes the steps of labeling the secreted proteins with a detectable label, such as biotin or ^{125}I, immunoprecipitating the M-CSF with an anti-M-CSF antibody and then detecting the labeled M-CSF. Another immunoassay for determining secreted levels of M-CSF can include the steps of (a) pre-binding anti-M-CSF antibodies to the surface of a microtiter plate; (b) adding tissue culture cell media or body fluid containing the secreted M-CSF to the wells of the microtiter plate to bind to the anti-M-CSF antibodies; (c) adding an antibody that will detect the anti-M-CSF antibody, e.g., anti-M-CSF labeled with digoxigenin that binds to an epitope of M-CSF different from the anti-M-CSF antibody of step (a); (d) adding an antibody to digoxigenin conjugated to peroxidase; and (e) adding a peroxidase substrate that will yield a colored reaction product that can be quantitated to determine the level of secreted M-CSF in tissue culture cell media or a body fluid sample. Methods such as ELISA, RIA, Western blot, immunohistochemistry, and antigen capture assay are well known in the art. See, e.g., Harlow and Lane, *supra*. In addition, the immunoassays can be scaled up for high throughput screening in order to test a large number of compounds for inhibition or activation of M-CSF.

[0257] The anti-M-CSF antibodies of the invention can also be used to determine the levels of cell surface M-CSF in a tissue or in cells derived from the tissue. In some embodiments, the tissue is from a diseased tissue. In some embodiments, the tissue can be a tumor or a biopsy thereof. In some embodiments of the method, a tissue or a biopsy thereof can be excised from a patient. The tissue or biopsy can then be used in an immunoassay to determine, e.g., total M-CSF levels, cell surface levels of M-CSF, or localization of M-CSF by the methods discussed above.

[0258] The method can comprise the steps of administering a detectably labeled anti-M-CSF antibody or a composition comprising them to a patient in need of

such a diagnostic test and subjecting the patient to imaging analysis to determine the location of the M-CSF-expressing tissues. Imaging analysis is well known in the medical art, and includes, without limitation, x-ray analysis, magnetic resonance imaging (MRI) or computed tomography (CE). The antibody can be labeled with any agent suitable for *in vivo* imaging, for example a contrast agent, such as barium, which can be used for x-ray analysis, or a magnetic contrast agent, such as a gadolinium chelate, which can be used for MRI or CE. Other labeling agents include, without limitation, radioisotopes, such as ^{99}Tc. In another embodiment, the anti-M-CSF antibody will be unlabeled and will be imaged by administering a second antibody or other molecule that is detectable and that can bind the anti-M-CSF antibody. In an embodiment, a biopsy is obtained from the patient to determine whether the tissue of interest expresses M-CSF.

[0259] The anti-M-CSF antibodies of the invention can also be used to determine the secreted levels of M-CSF in a body fluid such as blood serum, urine, or synovial fluid derived from a tissue. In some embodiments, the body fluid is from a diseased tissue. In some embodiments, the body fluid is from a tumor or a biopsy thereof. In some embodiments of the method, body fluid is removed from a patient. The body fluid is then used in an immunoassay to determine secreted M-CSF levels by the methods discussed above. One embodiment of the invention is a method of assaying for the activity of a M-CSF antagonist comprising: administering a M-CSF antagonist to a primate or human subject and measuring the number of CD14+CD16+ monocytes in a biological sample.

Therapeutic Methods of Use

[0260] In another embodiment, the invention provides a method for inhibiting M-CSF activity by administering an anti-M-CSF antibody to a patient in need thereof. Any of the types of antibodies described herein may be used therapeutically. In a preferred embodiment, the anti-M-CSF antibody is a human, chimeric or humanized antibody. In another preferred embodiment, the M-CSF is human and the patient is a human patient. Alternatively, the patient may be a mammal that expresses a M-CSF that the anti-M-CSF antibody cross-reacts with. The antibody may be administered to a non-human mammal expressing a M-CSF

with which the antibody cross-reacts (i.e. a primate) for veterinary purposes or as an animal model of human disease. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of this invention.

[0261] As used herein, the term "a disorder in which M-CSF activity is detrimental" is intended to include diseases and other disorders in which the presence of high levels of M-CSF in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Such disorders may be evidenced, for example, by an increase in the levels of M-CSF secreted and/or on the cell surface or increased tyrosine autophosphorylation of *c-fms* in the affected cells or tissues of a subject suffering from the disorder. The increase in M-CSF levels may be detected, for example, using an anti-M-CSF antibody as described above.

[0262] In one embodiment, an anti-M-CSF antibody may be administered to a patient who has a *c-fms*-expressing tumor or a tumor that secretes M-CSF and/or that expresses M-CSF on its cell surface. Preferably, the tumor expresses a level of *c-fms* or M-CSF that is higher than a normal tissue. The tumor may be a solid tumor or may be a non-solid tumor, such as a lymphoma. In a more preferred embodiment, an anti-M-CSF antibody may be administered to a patient who has a *c-fms*-expressing tumor, a M-CSF-expressing tumor, or a tumor that secretes M-CSF that is cancerous. Further, the tumor may be cancerous. In an even more preferred embodiment, the tumor is a cancer of lung, breast, prostate or colon. In another preferred embodiment, the anti-M-CSF antibody administered to a patient results in M-CSF no longer bound to the *c-fms* receptor. In a highly preferred embodiment, the method causes the tumor not to increase in weight or volume or to decrease in weight or volume. In another embodiment, the method causes *c-fms* on tumor cells to not be bound by M-CSF. In another embodiment, the method causes M-CSF on tumor cells to not be bound to *c-fms*. In another embodiment, the method causes secreted M-CSF of the tumor cells to not be bound to *c-fms*. In a preferred embodiment, the antibody is selected from 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, 9.7.2, 9.7.2C-Ser, 9.14.4C-Ser, 8.10.3C-Ser, 8.10.3-CG2, 9.7.2-CG2, 9.7.2-CG4, 9.14.4-CG2, 9.14.4-CG4, 9.14.4-

Ser, 9.7.2-Ser, 8.10.3-Ser, 8.10.3-CG4, 8.10.3FG1 or 9.14.4G1, or comprises a heavy chain, light chain or antigen binding region thereof.

[0263] In another preferred embodiment, an anti-M-CSF antibody may be administered to a patient who expresses inappropriately high levels of M-CSF. It is known in the art that high-level expression of M-CSF can lead to a variety of common cancers. In one embodiment, said method relates to the treatment of cancer such as brain, squamous cell, bladder, gastric, pancreatic, breast, head, neck, esophageal, prostate, colorectal, lung, renal, kidney, ovarian, gynecological or thyroid cancer. Patients that can be treated with a compounds of the invention according to the methods of this invention include, for example, patients that have been diagnosed as having lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina or carcinoma of the vulva), Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors (e.g., sarcomas, carcinomas or lymphomas that are cancers of body tissues other than blood, bone marrow or the lymphatic system), solid tumors of childhood, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas). In a more preferred embodiment, the anti-M-CSF antibody is administered to a patient with breast cancer, prostate cancer, lung cancer or colon cancer. In an even more preferred embodiment, the method causes the cancer to stop proliferating abnormally, or not to increase in weight or volume or to decrease in weight or volume.

[0264] The antibody may be administered once, but more preferably is administered multiple times. For example, the antibody may be administered from three times daily to once every six months or longer. The administering may be on

a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may also be administered continuously via a minipump. The antibody may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The antibody may be administered at the site of the tumor or inflamed body part, into the tumor or inflamed body part, or at a site distant from the site of the tumor or inflamed body part. The antibody may be administered once, at least twice or for at least the period of time until the condition is treated, palliated or cured. The antibody generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume or until the inflamed body part is healed. The antibody will generally be administered as part of a pharmaceutical composition as described *supra*. The dosage of antibody will generally be in the range of 0.1-100 mg/kg, more preferably 0.5-50 mg/kg, more preferably 1-20 mg/kg, and even more preferably 1-10 mg/kg. The serum concentration of the antibody may be measured by any method known in the art.

[0265] In another aspect, the anti-M-CSF antibody may be co-administered with other therapeutic agents, such as anti-inflammatory agents, anti-coagulant agents, agents that will lower or reduce blood pressure, anti-neoplastic drugs or molecules, to a patient who has a hyperproliferative disorder, such as cancer or a tumor. In one aspect, the invention relates to a method for the treatment of the hyperproliferative disorder in a mammal comprising administering to said mammal a therapeutically effective amount of a compound of the invention in combination with an anti-tumor agent selected from the group consisting of, but not limited to, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating agents, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, kinase inhibitors, matrix metalloprotease inhibitors, genetic therapeutics and anti-androgens. In a more preferred embodiment, the antibody may be administered with an antineoplastic agent, such as adriamycin or taxol. In another preferred embodiment, the antibody

or combination therapy is administered along with radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy. In yet another preferred embodiment, the antibody will be administered with another antibody. For example, the anti-M-CSF antibody may be administered with an antibody or other agent that is known to inhibit tumor or cancer cell proliferation, e.g., an antibody or agent that inhibits erbB2 receptor, EGF-R, CD20 or VEGF.

[0266] Co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses administering a pharmaceutical composition comprising the anti-M-CSF antibody and the additional therapeutic agent and administering two or more separate pharmaceutical compositions, one comprising the anti-M-CSF antibody and the other(s) comprising the additional therapeutic agent(s). Further, although co-administration or combination therapy generally means that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses instances in which the antibody and additional therapeutic agents are administered at different times. For instance, the antibody may be administered once every three days, while the additional therapeutic agent is administered once daily. Alternatively, the antibody may be administered prior to or subsequent to treatment of the disorder with the additional therapeutic agent. Similarly, administration of the anti-M-CSF antibody may be administered prior to or subsequent to other therapy, such as radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy

[0267] The antibody and one or more additional therapeutic agents (the combination therapy) may be administered once, twice or at least the period of time until the condition is treated, palliated or cured. Preferably, the combination therapy is administered multiple times. The combination therapy may be administered from three times daily to once every six months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months, or may be administered continuously via a minipump. The combination therapy may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route.

The combination therapy may be administered at a site distant from the site of the tumor. The combination therapy generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume.

[0268] In a still further embodiment, the anti-M-CSF antibody is labeled with a radiolabel, an immunotoxin or a toxin, or is a fusion protein comprising a toxic peptide. The anti-M-CSF antibody or anti-M-CSF antibody fusion protein directs the radiolabel, immunotoxin, toxin or toxic peptide to the M-CSF-expressing cell. In a preferred embodiment, the radiolabel, immunotoxin, toxin or toxic peptide is internalized after the anti-M-CSF antibody binds to the M-CSF on the surface of the target cell.

[0269] In another aspect, the anti-M-CSF antibody may be used to treat noncancerous states in which high levels of M-CSF and/or M-CSF have been associated with the noncancerous state or disease. In one embodiment, the method comprises the step of administering an anti-M-CSF antibody to a patient who has a noncancerous pathological state caused or exacerbated by high levels of M-CSF and/or M-CSF levels or activity. In a more preferred embodiment, the anti-M-CSF antibody slows the progress of the noncancerous pathological state. In a more preferred embodiment, the anti-M-CSF antibody stops or reverses, at least in part, the noncancerous pathological state.

Gene Therapy

[0270] The nucleic acid molecules of the instant invention can be administered to a patient in need thereof via gene therapy. The therapy may be either *in vivo* or *ex vivo*. In a preferred embodiment, nucleic acid molecules encoding both a heavy chain and a light chain are administered to a patient. In a more preferred embodiment, the nucleic acid molecules are administered such that they are stably integrated into chromosomes of B cells because these cells are specialized for producing antibodies. In a preferred embodiment, precursor B cells are transfected or infected *ex vivo* and re-transplanted into a patient in need thereof. In another embodiment, precursor B cells or other cells are infected *in vivo* using a virus known to infect the cell type of interest. Typical vectors used for gene therapy include liposomes, plasmids and viral vectors. Exemplary viral vectors are

retroviruses, adenoviruses and adeno-associated viruses. After infection either *in vivo* or *ex vivo*, levels of antibody expression can be monitored by taking a sample from the treated patient and using any immunoassay known in the art or discussed herein.

[0271] In a preferred embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of an anti-M-CSF antibody and expressing the nucleic acid molecule. In another embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an anti-M-CSF antibody and expressing the nucleic acid molecule. In a more preferred method, the gene therapy method comprises the steps of administering of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof and an isolated nucleic acid molecule encoding the light chain or the antigen-binding portion thereof of an anti-M-CSF antibody of the invention and expressing the nucleic acid molecules. The gene therapy method may also comprise the step of administering another anti-cancer agent, such as taxol or adriamycin.

[0272] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

EXAMPLE I

Generation of Cell Lines Producing Anti-M-CSF Antibody

[0273] Antibodies of the invention were prepared, selected, and assayed as follows:

Immunization and hybridoma generation

Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with human M-CSF (10 μg/dose/mouse). This dose was repeated five to seven times over a three to eight week period. Four days before fusion, the mice were given a final injection of human M-CSF in PBS. The spleen and lymph node lymphocytes from immunized mice were fused with the non-secretory myeloma P3-X63-Ag8.653 cell line, and the fused cells were subjected to HAT selection as previously described (Galfre and Milstein, *Methods Enzymol.*

73:3-46, 1981). A panel of hybridomas all secreting M-CSF specific human IgG2 and IgG4 antibodies was recovered. Antibodies also were generated using XENOMAX™ technology as described in Babcook, J.S. *et al.*, *Proc. Natl. Acad. Sci.* USA 93:7843-48, 1996. Nine cell lines engineered to produce antibodies of the invention were selected for further study and designated 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4, 8.10.3 and 9.7.2. The hybridomas were deposited under terms in accordance with the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209 on August 8, 2003. The hybridomas have been assigned the following accession numbers:

Hybridoma 3.8.3 (LN 15891)	PTA-5390
Hybridoma 2.7.3 (LN 15892)	PTA-5391
Hybridoma 1.120.1 (LN 15893)	PTA-5392
Hybridoma 9.7.2 (LN 15894)	PTA-5393
Hybridoma 9.14.4 (LN 15895)	PTA-5394
Hybridoma 8.10.3 (LN 15896)	PTA-5395
Hybridoma 88-gamma (UC 25489)	PTA-5396
Hybridoma 88-kappa (UC 25490)	PTA-5397
Hybridoma 100-gamma (UC 25491)	PTA-5398
Hybridoma 100-kappa (UC 25492)	PTA-5399
Hybridoma 252-gamma (UC 25493)	PTA-5400
Hybridoma 252-kappa (UC 25494)	PTA-5401

EXAMPLE II

Gene Utilization Analysis

[0274] DNA encoding the heavy and light chains of monoclonal antibodies 252, 88, 100, 3.8.3, 2.7.3, 1,120.1, 9.14.4, 8.10.3 and 9.7.2 was cloned from the respective hybridoma cell lines and the DNA sequences were determined by methods known to one skilled in the art. Additionally, DNA from the hybridoma cell lines 9.14.4, 8.10.3 and 9.7.2 was mutated at specific framework regions in the variable domain and/or isotype-switched to obtain, for example, 9.14.4I, 8.10.3F, and 9.7.2IF, respectively. From nucleic acid sequence and predicted amino acid sequence of the antibodies, the identity of the gene usage for each antibody chain was determined ("VBASE"). Table 2 sets forth the gene utilization of selected antibodies in accordance with the invention:

Table 2

Heavy and Light Chain Gene Utilization

Clone	Heavy Chain				Kappa Light Chain		
	SEQ ID NO:	V_H	D_H	J_H	SEQ ID NO:	V_κ	J_κ
252	1, 2	3-11	7-27	6	3, 4	O12	3
88	5, 6	3-7	6-13	4	7, 8	O12	3
100	9, 10	3-23	1-26	4	11, 12	L2	3
3.8.3	14	3-11	7-27	4	16	L5	3
2.7.3	18	3-33	1-26	4	20	L5	4
1.120.1	22	1-18	4-23	4	24	B3	1
9.14.4I	25, 26	3-11	7-27	4b	27, 28	O12	3
8.10.3F	29, 30	3-48	1-26	4b	31, 32	A27	4
9.7.2IF	33, 34	3-11	6-13	6b	35, 36	O12	3
9.14.4	37, 38	3-11	7-27	4b	27, 28	O12	3
8.10.3	29, 30	3-48	1-26	4b	43, 44	A27	4
9.7.2	45, 46	3-11	6-13	6b	47, 48	O12	3
8.10.3FG1	97, 98	3-48	1-26	4b	31, 32	A27	4
9.14.4G1	101, 102	3-11	7-27	4b	27, 28	O12	3
9.14.4C-Ser	54	3-11	7-27	4b	56	O12	3
9.14.4-CG2	74	3-11	7-27	4b	56	O12	3
9.14.4-CG4	78	3-11	7-27	4b	56	O12	3
8.10.3C-Ser	58	3-48	1-26	4b	60	A27	4
8.10.3-CG2	62	3-48	1-26	4b	60	A27	4
8.10.3-CG4	94	3-48	1-26	4b	60	A27	4
8.10.3-Ser	90	3-48	1-26	4b	43, 44	A27	4
9.7.2C-Ser	50	3-11	6-13	6b	52	O12	3
9.7.2-CG2	66	3-11	6-13	6b	52	O12	3
9.7.2-CG4	70	3-11	6-13	6b	52	O12	3
9.7.2-Ser	86	3-11	6-13	6b	47, 48	O12	3
9.14.4-Ser	82	3-11	7-27	4b	27, 28	O12	3

[0275] Mutagenesis of specific residues of the heavy and light chains was carried out by designing primers and using the QuickChange Site Directed Mutagenesis Kit from Stratagene, according to the manufacturer's instructions. Mutations were confirmed by automated sequencing, and mutagenized inserts were subcloned into expression vectors. The expression vectors were transfected into HEK293 cells to produce enough of the antibodies for characterization.

EXAMPLE III

M-CSF Mouse Monocytic Cell Proliferation Assay

[0276] *In vitro* assays were conducted to measure M-CSF-dependent mouse monocytic cell proliferation in the presence of anti-M-CSF antibodies to determine the degree of inhibition by anti-M-CSF antibodies.

[0277] Mouse monocytic cells, M-NFS-60 cells, from American Type Culture Collection (ATCC) (Manassas, VA), were obtained and maintained in RPMI-1640 medium containing 2 mM L-glutamine (ATCC), 10% heat inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA), 0.05 mM 2-mercaptoethanol (Sigma, St. Louis MO) (assay medium), with 15 ng/ml human M-CSF. M-NSF-60 cells were split to 5×10^4 for next day use or to 2.5×10^4 for use in 2 days. Prior to use in the assay, the cells were washed three times with RPMI-1640, counted and the volume adjusted with assay medium to yield 2×10^5 cells/ml. All conditions were conducted in triplicate in 96-well treated tissue culture plates (Corning, Corning, NY). To each well 50 μl of the washed cells, either 100 pM or 1000 pM M-CSF in a volume of 25 μl and test or control antibody at various concentrations in a volume of 25 μl in acetate buffer (140 mM sodium chloride, 20 mM sodium acetate, and 0.2 mg/ml polysorbate 80, pH 5.5) to a final volume of 100 μl was added. Antibodies of the invention were tested alone and with human M-CFS. The plates were incubated for 24 hours (hrs) at 37°C with 5% CO_2.

[0278] After 24 hrs, 10 μl/well of 0.5 μCi ^{3}H-thymidine (Amersham Biosciences, Piscataway, NJ) was added and pulsed with the cells for 3 hrs. To detect the amount of incorporated thymidine, the cells were harvested onto pre-wet unifilter GF/C filterplates (Packard, Meriden, CT) and washed 10 times with water. The

plates were allowed to dry overnight. Bottom seals were added to the filterplates. Next, 45 μl Microscint 20 (Packard, Meriden, CT) per well was added. After a top seal was added, the plates were counted in a Trilux microbeta counter (Wallac, Norton, OH).

[0279] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit mouse monocytic cell proliferation in response to M-CSF. Further, by using various concentrations of antibodies, the IC_{50} for inhibition of mouse nonocytic cell proliferation was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Cell Proliferation Assay, Table 3a and Table 3b).

Table 3a

Antibody	252	88	100	3.8.3	2.7.3	1.120.1
M-CSF Mouse Monocytic Cell Proliferation Assay [IC_{50}, M]	1.86 x 10^{-10}	2.31 x 10^{-10}	7.44 x 10^{-10}	7.3 x 10^{-11}	1.96 x 10^{-10}	1.99 x 10^{-10}
Human Whole Blood Monocyte Activation Assay [IC_{50}, M]	8.67 x 10^{-10}	5.80 x 10^{-10}	1.53 x 10^{-10}	8.6 x 10^{-11}	7.15 x 10^{-10}	8.85 x 10^{-10}
Receptor Binding Inhibition Assay [IC_{50}, M]	7.47 x 10^{-10}	4.45 x 10^{-10}	1.252 x 10^{-9}	7.0 x 10^{-11}	3.08 x 10^{-10}	1.57 x 10^{-10}

Table 3b

Antibody	9.14.4I	8.10.3F	9.7.2IF	9.14.4	8.10.3	9.7.2
M-CSF Mouse Monocytic Cell Proliferation Assay [IC_{50}, M]	2.02 x 10^{-10}	4.13 x 10^{-10}	7.37 x 10^{-10}	2.02 x 10^{-10}	4.13 x 10^{-10}	7.37 x 10^{-10}
Human Whole Blood Monocyte Activation Assay [IC_{50}, M]	2.49 x 10^{-10}	4.46 x 10^{-10}	1.125 x 10^{-9}	6.48 x 10^{-10}	2.8 x 10^{-10}	1.98 x 10^{-10}
Receptor Binding Inhibition Assay [IC_{50}, M]	2.97 x 10^{-10}	9.8 x 10^{-11}	5.29 x 10^{-10}	4.1 x 10^{-11}	1.5 x 10^{-9}	6 x 10^{-12}

EXAMPLE IV

Human Whole Blood Monocyte Activation Assay

[0280] *In vitro* assays were conducted to measure M-CSF dependent monocyte shape changes in the presence of anti-M-CSF antibodies to determine if the anti-M-CSF antibodies were capable of inhibiting whole blood monocyte activation and their degree of inhibition of monocyte shape changes.

[0281] In individual wells of a 96-well tissue culture plate, 6 μl of 1.7 nM anti-M-CSF and 94 μl of whole human blood for a final concentration of 102 pM anti-M-CSF antibody were mixed. The plates were incubated at 37°C in a CO_2 tissue

culture incubator. Next, the plates were removed from the incubator. To each well, 100 μl of a fixative solution (0.5% formalin in phosphate buffered saline without $MgCl_2$ or $CaCl_2$) was added and the plates were incubated for 10 minutes at room temperature. For each sample, 180 μl from each well and 1 ml of Red Cell Lysis Buffer were mixed. The tubes were vortexed for 2 seconds. Next, the samples were incubated at 37°C for 5 minutes in a shaking water bath to lyse the red blood cells, but to leave monocytes intact. Immediately following this incubation, the samples were read on a fluorescence-activated cell scanning (FACS) machine (BD Beckman FACS) and data was analyzed using FACS Station Software Version 3.4.

[0282] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit monocyte shape changes compared to control samples. Using the monocyte shape change assay, the IC_{50} was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Human Whole Blood Monocyte Activation, Table 3a and Table 3b).

EXAMPLE V

c-fms Receptor Binding Inhibition Assay

[0283] *In vitro* assays were conducted to measure M-CSF binding to *c-fms* receptor in the presence of anti-M-CSF antibodies to determine if the anti-M-CSF antibodies were capable of inhibiting M-CSF binding to *c-fms* receptor and their degree of inhibition.

[0284] NIH-3T3 cells transfected with human *c-fms* or M-NSF-60 cells maintained in Dulbecco's phosphate buffered saline without magnesium or calcium were washed. NIH-3T3 cells were removed from tissue culture plates with 5 mM ethylene-diamine-tetra-acetate (EDTA), pH 7.4. The NIH-3T3 cells were returned to the tissue culture incubator for 1-2 minutes and the flask(s) were tapped to loosen the cells. The NIH-3T3 cells and the M-NSF-60 cells were transferred to 50 ml tubes and washed twice with reaction buffer (1x RPMI without sodium bicarbonate containing 50 mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4). Next, the NIH-3T3 cells were resuspended

in reaction buffer for a final concentration of 1.5 x 10^5 cell/ml. The M-NSF-60 cells were resuspended in a reaction buffer for a final concentration of 2.5 x 10^6 cells/ml.

[0285] For the assay, 9 μl of a sterile 0.4 M sucrose solution, 100 μl of ^{125}I-M-CSF (Amersham, IMQ7228v) at a final concentration of 200 pM in RPMI-1640 containing 50 mM HEPES (pH 7.4), 0.2% bovine serum albumin, and 100 μl of unlabeled M-CSF at a final concentration of 200 nM were mixed in a binding tube. Next, 50 μl/tube of increasing concentrations of a test antibody was added. In order to determine non-specific binding of the antibodies, we included samples to which we also added 200 nM M-CSF. To control tubes, we did not add antibody. Next, 15,000 NIH-3T3 cells or 250,000 M-NSF-60 cells were added per tube. All tubes were incubated at room temperature for 3 hrs and subjected to centrifugation at 10,000 rpm for 2 min. The tips of the tubes containing the cell pellets were cut off and the amount of M-CSF bound to the cells was determined using a Packard Cobra II Gamma counter. The specific binding was determined by subtracting non-specific binding from total binding. All assays were performed in duplicate. The binding data was analyzed using the computer program, Graph Pad Prism 2.01.

[0286] These experiments demonstrate that anti-M-CSF antibodies of the invention inhibit the binding of M-CSF to *c-fms* receptor compared to control samples. Further, by using various concentrations of antibodies, the IC_{50} for inhibition of receptor binding was determined for antibodies 252, 88, 100, 3.8.3, 2.7.3, 1.120.1, 9.14.4I, 8.10.3F, 9.7.2IF, 9.14.4, 8.10.3, and 9.7.2 (Receptor Binding Inhibition Assay, Table 3a and Table 3b).

EXAMPLE VI

Determination of Affinity Constants (K_D) of Anti-M-CSF Monoclonal Antibodies by BIACORE™

[0287] Affinity measures of purified antibodies were performed by surface plasmon resonance using the BIACORE™ 3000 instrument, following the manufacturer's protocols.

[0288] For antibodies 3.8.3, 2.7.3 and 1.120.1, the experiments were performed in a BIACORE™ 3000 instrument at 25°C in Dulbecco's phosphate buffered saline containing 0.0005% Tween-20. Protein concentrations were obtained from sedimentation velocity experiments or by measuring the wavelength of the sample at 280 nm using theoretical extinction coefficients derived from amino acid sequences. For experiments measuring the binding of antibody to immobilized antigens, M-CSF was immobilized on a B1 chip by standard direct amine coupling procedures. Antibody samples were prepared at 0.69 μM for 3.8.3, 2.7.3 and 1.120.1. These samples were diluted 3-fold serially to 8.5 nM or 2.8 nM for roughly a 100-fold range in concentrations. For each concentration, the samples were injected in duplicate at 5 μl/min flow for 4 min. The dissociation was monitored for 2000 seconds. The data were fit globally to a simple 1:1 binding model using BIACORE™ Biaevaluation software. In all cases, this method was used to obtain k_{off} and it was found that this data set compared well to data obtained from global fit of association and dissociation data.

[0289] For antibodies 252, 88 and 100, the experiments were performed in a BIACORE™ 3000 instrument at 25°C in HBS-EP Buffer (0.01M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20). For experiments measuring the binding of antibody to immobilized antigens, a M-CSF was immobilized on a CM5 Research Grade Sensor chip by standard direct amine coupling procedures. Antibody samples were prepared at 12.5 nM for antibodies 252 and 100 and at 25.0 nM for antibody 88. These samples were two-fold serially diluted to 0.78 nM for roughly a 15-30 fold range in concentrations. For each concentration, the samples were injected in duplicate in random order at 30 μl/min flow for 3 min. The dissociation was monitored for 300 sec. The data were fit globally to a simple 1:1 binding model using BIACORE™ Biaevaluation software. In all cases, this method was used to obtain k_{off} and it was found that this data set compared well to data obtained from global fit of association and dissociation data.

[0290] Table 4 shows results for antibodies 252, 88, 100, 3.8.3, 2.7.3 and 1.120.1.

Table 4

	252	88	100	3.8.3	2.7.3	1.120.1
K_D (M)	1.33×10^{-11}	1.33×10^{-9}	2.0×10^{-11}	4.0×10^{-10}	4.7×10^{-9}	5.4×10^{-9}
k_{off} (1/s)	1.03×10^{-6}	7.3×10^{-5}	1.7×10^{-5}			

EXAMPLE VII

Production of 8.10.3 antibodies from 8.10.3 hybridoma cells

[0291] Antibody 8.10.3 was produced in 3L sparged spinners. The 3L sparged spinner flask is a glass vessel where cultures are mixed with an impeller controlled by a magnetic platform. The spinner is connected to gas lines to provide 5% CO_2 and air. 8.10.3 hybridoma cells were initially thawed into T-25 cell culture flasks. The cells were progressively expanded until there was a sufficient number of cells to seed the sparged spinners.

[0292] Two 3L sparged spinner flasks were seeded with 8.10.3 hybridoma cells in Hybridoma Serum-Free Medium with the additions noted on Table 5, for the two sparged flasks. The concentrations for Ultra low IgG serum (Gibco cat# 16250-078), L-glutamine (JRH Biosciences cat# 59202-500M), Non-Essential Amino Acids (Gibco cat# 11140-050), Peptone (Difco cat# 211693), glucose (In-house stock prepared from JT Baker cat# 1920-07), and Anti-foam C (Sigma cat.# A-8011) are given at their final concentrations in the media. The balance of the volume in each reactor is Hybridoma Serum-Free Medium.

Table 5. Conditions for Growing Hybridoma 8.10.3 in two 3L sparged spinners.

Conditions	**Spinner 1**	**Spinner 2**
Seeding density (1×10^6 cells/ml)	0.16 ml	0.16 ml
Hybridoma Serum-Free Medium (Gibco cat# 12045-076)	Balance	Balance
Ultra low IgG serum (Gibco cat# 16250-078)	5%	5%
L-glutamine (JRH Biosciences cat# 59202-500M)	8 mmol/L	8mmol/L

Conditions	Spinner 1	Spinner 2
Non-Essential Amino Acids (Gibco cat# 11140-050)	1%	1%
Peptone (Difco cat# 211693)	1g/L	1g/L
2M glucose (In-house stock prepared from JT Baker cat# 1920-07)	8g/L	8g/L
Anti-foam C (Sigma cat.# A-8011)	1ml/L	1ml/L

[0293] The cultures were grown for 15 days and were harvested when the viability was below 20%. Viability was determined by trypan blue exclusion method with an automated cell counter (Cedex, Innovatis). Harvesting was accomplished by centrifugation and subsequent filtration. Clarified supernatant was obtained after centrifugation for 15 minutes at 7000 rpm and subsequent filtration with a sterile 0.22 μm 4" Opticap Millipore filter (cat# KVSCO4HB3) into a 10L sterile TC-Tech bag (cat # P/N 12420 Bag Style CC-10-112420). The filtrate was then purified in the following example.

EXAMPLE VIII

Purification of an Anti-M-CSF Antibody

[0294] A Protein A column (Amersham Pharmacia) was prepped by washing with 3 column volumes of 8M Urea, followed by an equilibration wash with 20 mM Tris (pH 8). The final filtrate from Example VII was spiked with 2% v/v of 1M Tris pH 8.3 and 0.02% NaN_3 before being loaded onto the Protein A column via gravity-drip mode. After load was complete, the resin was washed with 5 column volumes of 20 mM Tris (pH 8), followed by 5 column volumes of the elution buffer (0.1 M Glycine pH 3.0). Any precipitation was noted, and then a 10% v/v spike of 1M Tris pH 8.3 was added to the eluted antibody. The eluted protein was then dialyzed into 100 fold the volume amount of eluted material of dialysis buffer (140 mM NaCl/20mM Sodium Acetate pH 5.5). Following dialysis, the antibody was sterile filtered with a 0.22 μm filter and stored until further use.

EXAMPLE IX

Monkey Treatment and Monocyte Counts

[0295] One male and one female cynomolgus monkey per dosage group were intravenously administered vehicle or antibody 8.10.3 (produced as describe in Examples VII and VIII) at 0, 0.1, 1, or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. Blood samples for clinical laboratory analysis were collected at 24 and 72 hours postdose and weekly for 3 weeks. The monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system (Abbott Park, Illinois).

[0296] A dose-related decrease (~25% to 85%) in total monocytes at all doses (Figures 1A and 1B) was observed. Monocyte counts at the 0.1 and 1 mg/kg appeared to rebound to near control levels by week 2, while monocyte counts at 5 mg/kg were still decreased at 3 weeks.

CD14+CD16+ monocyte subset analysis

[0297] Primate whole blood was drawn into Vacutainer tubes containing sodium heparin. 0.2 ml of each blood sample was added to a 15 ml conical polypropylene centrifuge tube containing 10 ml of red blood cell lysis buffer (Sigma), and incubated in a 37°C water bath for 15 minutes. The tubes were then centrifuged in a Sorvall RT7 centrifuge for 5 minutes at 1,200 rpm. The supernatant was aspirated, the pellet resuspended in 10 ml of 4°C FACS buffer (Hanks' Balanced Salt Solution/2%FBS/0.02% sodium azide), and the tube centrifuged again for 5 minutes at 1,200 rpm. The supernatant was aspirated and the pellet resuspended in an antibody cocktail consisting of 80 µl 4°C FACS buffer, 10 µl FITC-conjugated anti-human CD14 monoclonal antibody (BD Biosciences, San Diego, CA), 0.5 µl Cy5-PE-conjugated anti-human CD16 monoclonal antibody (BD Biosciences, San Diego, CA), and 10 µl PE-conjugated anti-human CD89 monoclonal antibody (BD Biosciences, San Diego, CA). The cell suspension was incubated on ice for 20 minutes, after which 10 ml of 4°C FACS buffer was added and the cells centrifuged as before. The supernatant was aspirated, and the cell pellet resuspended in 400 µl FACS buffer and the cells analyzed on a FACSCaliber flow

cytometer (BD Biosciences, San Jose, CA). Data for 30,000 cells were collected from each sample.

[0298] The monocyte population was identified by a combination of forward angle light scatter and orthogonal light scatter. Cells within the monocyte gate were further analyzed for expression of CD14 and CD16. Two distinct population of monocytes were observed, one expressing high levels of CD14 with little or no CD16 expression (CD14++CD16-) and the other expressing lower levels of CD14, but high levels of CD16 (CD14+CD16+), similar to the two monocyte subsets previously described in human peripheral blood (Ziegler-Heitbrock H.W., *Immunology Today* 17:424-428 (1996)). For each primate tested, the percentage of monocytes within the CD14+CD16+ subset was determined after each blood draw, on days 1, 3, 7, 14, and 21 after 8.10.3 injection.

[0299] In general, 8.10.3 treatment resulted in a reduction in the percentage of CD14+CD16+ monocytes (see Figures 2A and 2B). Monkeys not receiving 8.10.3 Antibody demonstrated relatively stable CD14+CD16+ monocyte levels. CD14+CD16+ monocytes have been termed "proinflammatory" because they produce higher levels of TNF-α and other inflammatory cytokines (Frankenberger, M.T., *et al.*, *Blood* 87:373-377 (1996)). It has also been reported that the differentiation of monocytes from the conventional CD14++CD16- phenotype to the proinflammatory phenotype is dependent on M-CSF (Saleh M.N., *et al.*, *Blood* 85: 2910-2917 (1995)).

EXAMPLE X

Monkey Treatment and Monocyte Counts

[0300] Three male cynomolgus monkeys per dosage group were intravenously administered vehicle (20 mM Sodium acetate, pH 5.5, 140 mM NaCl), purified antibody 8.10.3F, or purified antibody 9.14.4I at 0, 1, or 5 mg/kg in a dose volume of 3.79 mL/kg over an approximately 5 minute period. The monkeys were 4 to 9 years of age and weighed 6 to 10 kg. Blood samples for clinical laboratory analysis were collected at 2, 4, 8, 15, 23, and 29 days. Monocyte counts were determined by light scatter using an Abbott Diagnostics Inc. Cell Dyn system (Abbott Park, Illinois).

[0301] A decrease in the percentage change in total monocytes at all doses of antibody 8.10.3F and antibody 9.14.4I as compared to pre-test levels of monocytes (Figures 3A and 3B) was observed (see e.g., day 4, 8, 15, and 23 in Figures 3A and 3B).

[0302] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCES

Key:

Signal peptide: underlined lower case
CDRs 1,2,3: underlined UPPERCASE
Variable domain: UPPER CASE
Constant domain: lower case
Mutations from germline in bold

SEQ ID NO: 1
252 Heavy Chain [Gamma chain] nucleotide sequence

atggagttggggctgtgctggattttccttgttgctattataaaaggtgtccagtgtCAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGATTTCATACATTAGTGGTA
GTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCAT
CTCCAGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCT
GAGAGCCGAGGACACGGCCGTGTATCACTGTGCGAGAGCCCTGGGTGG
GATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCTtcca
ccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttccc
agctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagac
ctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagt
gcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttcccccccaaaacccaaggacaccctcatga
tctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactggtac
gtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtca
gcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctccca
gccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgccccatccc
gggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggct
ctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 2
252 Heavy Chain [Gamma chain] protein sequence

melglcwiflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIR
QAPGKGLEWISYISGSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRAE
DTAVYHCARALGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfp
epvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcp
appvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsv
ltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiave
wesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 3
252 Light Chain [Kappa chain] nucleotide sequence
atgagggtccctgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCCAGAT
GACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCATTAGCGGCTTTTTAAATTGGTATC
AGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTACATCCA
GTTTGCAAAGTGGGGTCCCATTCAGGTTCAGTGGCAGTGGATCTGGGA
CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAAC
TTATTACTGTCAACAGAGTTACAGTGTCCCATTCACTTTCGGCCCTGGG
ACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagc
agttgaaatctggaactgctagcgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggt
ggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctc
agcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcc
tgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 4
252 Light Chain [Kappa chain] protein sequence
mrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSISGFLNWYQQK
PGKAPKLLIYATSSLQSGVPFRFSGSGSGTDFTLTISSLQPEDFATYYCQQS
YSVPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgns
qesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 5
88 Heavy Chain [Gamma chain] nucleotide sequence
atggaatttgggctgtgctgggttttccttgttgctattttagaaggtgtccagtgtGAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGAGCTGGGTCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAAGCAA
GATGGAAGTGAGAAATACTATGTGGACTCTGTGAAGGGCCGATTCACC
ATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCTCCGGGTATAGCA
GCAGCTGGTAGGGCCTACTGGGGCCAGGGAACCCTGGTCACCGTCTCC
TCAGCTtccaccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggc
cctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcg
tgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttc
ggcacccagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaat
gttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttcccccccaaaacccaagg
acaccctcatgatctcccggaccccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtcca
gttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacg
ttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaac
aaaggcctcccagccccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacacc
ctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcg
acatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccg
acggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccg
tgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 6
88 Heavy Chain [Gamma chain] protein sequence
mefglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWV
RQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSL
RAEDTAVYYCAPGIAAAGRAYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccv
ecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfns
tfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfyp
sdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 7
88 Light Chain [Kappa chain] nucleotide sequence
atgagggtccctgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCCAGAT
GACCCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGAGACAGAGTCACC
ATCACTTGCCGGCCAAGTCAGGACATTAGCAGTTATTTAAATTGGTATC
AGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCA
GTTTGCAAAGTGGGGTCCCATTAAGGTTCAGTGGCAGTGGATCTGGGA
CAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAAC
TTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCTGGG
ACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagc
agttgaaatctggaactgctagcgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggt
ggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctc
agcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcc
tgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 8
88 Light Chain [Kappa chain] protein sequence
mrvpaqllglllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQDISSYLNWYQQK
PGKAPKLLIYAASSLQSGVPLRFSGSGSGTDFTLTISSLQPEDFATYYCQQS
YSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgns
qesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 9
100 Heavy Chain [Gamma chain] nucleotide sequence
atggagtttgggctccgctggatttttcttgtggctattttaaaaggtgtccagtgtGAGGTGCAGCTGTTG
GAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCC
GCCAGGCTCCAGGGAAGGGGCTGGAATGGGTCTCAGCTATTAGTGGTC
GTGGTGGTAGGACATACTTCGCAGACTCCGTGAAGGGCCGGTTCACCA
TCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCC
TGAGAGCCGAGGACACGGCCGTATATTTCTGTGCGGGTAGAAGGCTATA
GTGGGCGCTACGGATTTTTTGACTACTGGGGCCAGGGAACCCTAGTCAC
CGTCTCCTCAGCCtccaccaagggcccatcggtcttcccccctggcgccctgctctagaagcacctccgag
agcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctct
gaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcc
ctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagaca
gttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaaga
ccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagca
gttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtg
caaggtctccaacaaaggcctcccagccccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggc
ttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctccca
tgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtc
ttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 10
100 Heavy Chain [Gamma chain] protein sequence
mefglrwiflvailkgvqcEVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVR
QAPGKGLEWVSAISGRGGRTYFADSVKGRFTISRDNSKNTLYLQMNSLRA
EDTAVYFCAVEGYSGRYGFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaal
gclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkc
cvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqf
nstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgf
ypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspg
k

SEQ ID NO: 11
100 Light Chain [Kappa chain] nucleotide sequence
atggaagccccagctcagcttctcttcctcctgctactctggctcccagataccactggaGAAATAGTGATG
ACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAACTTAGCCTGGTACC
AGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAC
CAGGGCCAGTGGTATCCCAGACAGGATCAGTGGCAGTGGGTCTGGAAC
AGAGTTCACTCTCATCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTT
TATTACTGTCAGCAGTCTAATAACTGGCCATTCACTTTCGGCCCTGGGA
CCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatgagca
gttgaaatctggaactgctagcgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtg
gataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctca
gcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcct
gagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 12
100 Light Chain [Kappa chain] protein sequence
meapaqllfllllwlpdttgEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQ
KPGQAPRLLIYGASTRASGIPDRISGSGSGTEFTLIISSLQSEDFAVYYCQQS
NNWPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgn
sqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 14
3.8.3 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWFSYISSSGSTIYYADSVKGRFTISRDNAKNSLSLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpa
ppvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvlt
vvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavew
esngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 16
3.8.3 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTISCRASQDISGWLAWY
QQKPGKAPKLLISATSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQTNSFPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalq
sgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 18
2.7.3 Heavy Chain [Gamma chain] protein sequence
mefglswvflvallrgcqcQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV
RQAPGKGLEWVAFIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSL
RAEDTAVYYCARGYRVYFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgcl
vkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygp
pcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfns
tyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfy
psdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 20
2.7.3 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwfpgsrcDIQMTQSPSSVSASVGDRVTITCRASQDISSWLAWY
QRKPGKAPKLQIYAASSLESGVPSRFNGSGSGTDFTLSISSLQPEDFATYYC
QQTNSFPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnal
qsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 22
1.120.1 Heavy Chain [Gamma chain] protein sequence
mewtwsflflvaaatgahsQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWV
RQAPGQGLEWMGWISAYNGNTNYAQKLQDRVTMTTDTSTTTAYMELRS
LRSDDTAVYYCARRAYGANFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaa
lgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverk
ccvecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpree
qfnstfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvk
gfypsdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls
pgk

SEQ ID NO: 24
1.120.1 Light Chain [Kappa chain] protein sequence
mvlqtqvfislllwisgaygDIVMTQSPDSLAVSLGERATINCKSSQSILFFSNNKNYL
AWYRQKPGQPPNLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVA
VYYCQQYYSSPWTFGQGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvq
wkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 25
9.14.4I Heavy Chain [Gamma Chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGAGCTGGA
TCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTA
GTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCA
CCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACA
GCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAA
CTGGGGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTtcc
accaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcct
ggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcc
cagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccaga
cctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgag
tgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatg
atctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactggta
cgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtc
agcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctccc
agcccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcc
cgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtgg
agtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttc
ctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggc
tctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 26
9.14.4I Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpa
ppvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvlt
vvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavew
esngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 27
9.14.4, 9.14.4I, 9.14.4-Ser and 9.14.4-G1 Light Chain [Kappa Chain] nucleotide sequence

atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgTGACATCC
AGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTCGGAGACAGAGT
CACCATCACTTGCCGGCCAAGTCAGATCATTAGCAGTTTATTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCCATGCTGCA
TCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTG
GGACAGATTTCACTCTCACCATCAGTAGTCTGCAACCTGAAGATTTTGC
AACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCT
GGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgga
aggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctaca
gcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatca
gggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 28
9.14.4, 9.14.4I, 9.14.4-Ser and 9.14.4-G1 Light Chain [Kappa Chain] protein sequence

mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQIISSLLNWYQ
QKPGKAPKLLIHAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqs
gnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 37
9.14.4 Heavy Chain [Gamma Chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtCCAGTGTCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGAGCTGGA
TCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTA
GTAGTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCA
CCATCTCCAGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACA
GCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAA
CTGGGGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTtcc
accaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcct
ggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttcc
cagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacgaaga
cctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagagagttgagtccaaatatggtcccca
tgcccatcatgcccagcacctgagttcctgggggaccatcagtcttcctgttcccccaaaacccaaggacactctca
tgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgaggtccagttcaactgg
tacgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaacagcacgtaccgtgtgg
tcagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctc
ccgtcctccatcgagaaaaccatctccaaagccaaagggcagccccgagagccacaggtgtacaccctgcccccat
cccaggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgt
ggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttc
ttcctctacagcaggctaaccgtggacaagagcaggtggcaggaggggaatgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacacagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 38
9.14.4 Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcpscpa
peflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvl
tvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiave
wesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 54
9.14.4C-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcppcpa
peflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvl
tvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiave
wesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 56
9.14.4C-Ser, 9.14.4-CG2 and 9.14.4-CG4 Light Chain [Kappa chain] protein sequence

mdmrvpaqllglllwlrgarcDIQMTQSPSSLSASVGDRVTITCRPSQIISSLLNWYQ
QKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqs
gnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 74
9.14.4-CG2 Heavy Chain [Gamma chain] protein sequence

mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcpa
ppvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsvlt
vvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiavew
esngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 78
9.14.4-CG4 Heavy Chain [Gamma chain] protein sequence

mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcpscpa
peflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvl
tvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiave
wesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 82
9.14.4-Ser Heavy Chain [Gamma chain] protein sequence

mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcp**p**cpa
peflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvsvl
tvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiave
wesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO. 101
9.14.4G1 Heavy chain (gamma chain) nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtCAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTGACTACTATATGAGCTGGATCC
GCCAGGCTCCAGGGAAGGGACTGGAGTGGGTTTCATACATTAGTAGTA
GTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCAT
CTCCAGGGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCT
GAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGCCTAACTGG
GGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCTtccaccaag
ggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctggtcaa
ggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctg
tcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacat
ctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcaca
catgcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacacc
ctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaa
ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccg
tgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaag
ccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcc
cccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcg
ccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggct
ccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgc
atgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatag

SEQ ID NO 102
9.14.4G1 Heavy chain (gamma chain) protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARGLTGDYWGQGTLVTVSSAstkgpsvfplapsskstsggtaalgclvkdyfp
epvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcpp
cpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrv
vsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdia
vewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 29
8.10.3 and 8.10.3F Heavy Chain [Gamma chain] nucleotide sequence
atggagttggggctgtgctgggttttccttgttgctattttagaaggtgtccagtgtGAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTAGTTTTAGTATGACCTGGGTCC
GCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTA
GAAGTAGTACCATATCCTACGCAGACTCTGTGAAGGGCCGATTCACCA
TCTCCAGAGACAATGCCAAGAACTCACTGTATCTGCAAATGAACAGCC
TGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCTCTTCT
AGCGGGAGCTACCTTCTTTGACTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCAGCCtccaccaagggcccatcggtcttcccctggcgccctgctccaggagcacctccgag
agcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctct
gaccagcggcgtgcacaccttcccagctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgcc
ctccagcaacttcggcacccagacctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagaca
gttgagcgcaaatgttgtgtcgagtgcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttccccc
caaaacccaaggacaccctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaaga
ccccgaggtccagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagca
gttcaacagcacgttccgtgtggtcagcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtg
caaggtctccaacaaaggcctcccagccccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaacc
acaggtgtacaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggc
ttctaccccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacacctccca
tgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtc
ttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 30
8.10.3 and 8.10.3F Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkcc
vecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfn
stfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfy
psdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 31
8.10.3FG1 and 8.10.3F Light Chain [Kappa chain] nucleotide sequence
atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTGTGTTG
ACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCC
TCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGTTACTTAGCCTGGTA
CCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCC
AGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGG
ACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAG
TGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACTTTCGGCGGAGG
GACCAAGGTGGAGATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatga
gcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaag
gtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcc
tcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcaggg
cctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 32
8.10.3FG1 and 8.10.3F Light Chain [Kappa chain] protein sequence
metpaqllfllllwlpdttgEFVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ
KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ
YGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsg
nsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 43
8.10.3 and 8.10.3-Ser Light Chain [Kappa chain] nucleotide sequence
atggaaaccccagcgcagcttctcttcctcctgctactctggctcccagataccaccggaGAATTTGTGTTG
ACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCC
TCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGTTACTTAGCCTGGTA
CCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCC
AGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGG
ACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGTAG
TGTATTACTGTCAGCAGTATGGTAGCTCACCTCTCACTTTCGGCGGAGG
GACCAAGGTGGAGATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctgatga
gcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaag
gtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcc
tcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcaggg
cctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 44
8.10.3 and 8.10.3-Ser Light Chain [Kappa chain] protein sequence
metpaqllfllllwlpdttgEFVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ
KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFVVYYCQQ
YGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsg
nsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 58
8.10.3C-Ser Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskyg
ppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqf
nstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgf
ypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 60
8.10.3-CG2, 8.10.3-CG4 and 8.10.3C-Ser Light Chain [kappa chain] protein sequence
metpaqllfllllwlpdttgEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ
KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ
YGSSPLTFGGGTKVEIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsg
nsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 62
8.10.3-CG2 Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkcc
vecppcpappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfn
stfrvvsvltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfy
psdiavewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 90
8.10.3-Ser Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskyg
ppcppcpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqf
nstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgf
ypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 94
8.10.3-CG4 Heavy Chain [Gamma chain] protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapcsrstsestaalg
clvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskyg
ppcpscpapeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqf
nstyrvvsvltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgf
ypsdiavewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 97
8.10.3FG1 Heavy Chain nucleotide sequence
atggagttggggctgagctgggttttccttgttgctattataaaaggtgtccagtgtGAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTAGTTTTAGTATGACCTGGGTCC
GCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTA
GAAGTAGTACCATATCCTACGCAGACTCTGTGAAGGGCCGATTCACCA
TCTCCAGAGACAATGCCAAGAACTCACTGTATCTGCAAATGAACAGCC
TGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGATCCTCTTCT
AGCGGGAGCTACCTTCTTTGACTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCAGCCtccaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctggg
ggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccc
tgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgc
cctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaa
agttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagt
cttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtg
agccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc
gggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaa
ggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcag
ccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcc
tggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagac
cacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagca
ggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccg
ggtaaatag

SEQ ID NO: 98
8.10.3FG1 Heavy chain (gamma chain) protein sequence
melglcwvflvailegvqcEVQLVESGGGLVQPGGSLRLSCAASGFTFSSFSMTWV
RQAPGKGLEWVSYISSRSSTISYADSVKGRFTISRDNAKNSLYLQMNSLRD
EDTAVYYCARDPLLAGATFFDYWGQGTLVTVSSAstkgpsvfplapssskstsggtaal
gclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepk
scdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpr
eeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsltclv
kgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsls
pgk

SEQ ID NO: 33
9.7.2IF Heavy Chain [Gamma chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtcAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTA
GTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCAT
CTCCAGGGACAACGCCAAGAATTCACTGTATCTGCAAATGAACAGCCT
GAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGGCGTATAGGAGG
TATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCTtcca
ccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttccc
agctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcaacttcggcacccagac
ctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagacagttgagcgcaaatgttgtgtcgagt
gcccaccgtgcccagcaccacctgtggcaggaccgtcagtcttcctcttccccccaaaacccaaggacaccctcatga
tctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccacgaagaccccgaggtccagttcaactggtac
gtggacggcgtggaggtgcataatgccaagacaaagccacgggaggagcagttcaacagcacgttccgtgtggtca
gcgtcctcaccgttgtgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctccca
gccccatcgagaaaaccatctccaaaaccaaagggcagccccgagaaccacaggtgtacaccctgccccccatccc
gggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgtgga
gtgggagagcaatgggcagccggagaacaactacaagaccacacctcccatgctggactccgacggctccttcttcc
tctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggct
ctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 34
9.7.2IF Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyf
pepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppc
pappvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvs
vltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiav
ewesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 35
9.7.2IF Light Chain [Kappa chain] nucleotide sequence
atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCC
AGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGT
CACCATCACTTGCCGGGCAAGTCAGAGCATTAGCGGCTTTTTAATTTGG
TATCAGCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTACA
TCCAGTTTACAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTG
GGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGC
AACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCT
GGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgga
aggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctaca
gcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatca
gggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 36
9.7.2IF Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSISGFLIWYQ
QRPGKAPKLLIYATSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqs
gnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 45
9.7.2 Heavy Chain [Gamma chain] nucleotide sequence
atggagtttgggctgagctgggttttccttgttgctattataaaaggtgtccagtgtcAGGTGCAGCTGGTG
GAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTCACCTTCAGTGACTACTACATGAGCTGGATCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGTA
GTGGTAGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTCACCAT
CTCCAGGGACAACGCCAAGAATTCACTGTATCTGCAAATGAACAGCCT
GAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGGCGTATAGGAGG
TATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAGCTtcca
ccaagggcccatccgtcttccccctggcgccctgctctagaagcacctccgagagcacagcggccctgggctgcctg
gtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgctctgaccagcggcgtgcacaccttccc
agctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacgaagac
ctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagagagttgagtccaaatatggtccccat
gcccatcatgcccagcacctgagttcctgggggggaccatcagtcttcctgttcccccaaaacccaaggacactctcat
gatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccaggaagaccccgaggtccagttcaactggt
acgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaacagcacgtaccgtgtggt
cagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagtacaagtgcaaggtctccaacaaaggcctc
ccgtcctccatcgagaaaaccatctccaaagccaaagggcagccccgagagccacaggtgtacaccctgccccat
cccaggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctaccccagcgacatcgccgt
ggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttc
ttcctctacagcaggctaaccgtggacaagagcaggtggcaggaggggaatgtcttctcatgctccgtgatgcatgag
gctctgcacaaccactacacacagaagagcctctccctgtctccgggtaaa

SEQ ID NO: 46
9.7.2 Heavy Chain [Gamma Chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyf
pepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcpsc
papeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvv
svltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdia
vewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 47
9.7.2 and 9.7.2-Ser Light Chain [Kappa chain] nucleotide sequence
atggacatgagggtccccgctcagctcctggggctcctgctactctggctccgaggtgccagatgtGACATCC
AGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGT
CACCATCACTTGCCGGGCAAGTCAGAGCATTAGCGGCTTTTTAATTTGG
TATCAGCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTACA
TCCAGTTTACAAAGTGGGGTCCCATTAAGGTTCAGTGGCAGTGAATCTG
GGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGC
AACTTACTACTGTCAACAGAGTTACAGTACCCCATTCACTTTCGGCCCT
GGGACCAAAGTGGATATCAAACGAactgtggctgcaccatctgtcttcatcttcccgccatctga
tgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgga
aggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctaca
gcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatca
gggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

SEQ ID NO: 48
9.7.2 and 9.7.2-Ser Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSISGFLIWYQ
QRPGKAPKLLIYATSSLQSGVPLRFSGSESGTDFTLTISSLQPEDFATYYCQ
QSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqs
gnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 50
9.7.2C-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCAIRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfp
epvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcppcp
apeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs
vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiav
ewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 52
9.7.2C-Ser, 9.7.2-CG2 and 9.7.2-CG4 Light Chain [Kappa chain] protein sequence
mdmrvpaqllgllllwlrgarcDIQMTQSPSSLSASVGDRVTITCRASQSISGFLIWYQ
QKPGKAPKLLIYATSSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
QSYSTPFTFGPGTKVDIKRtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqs
gnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

SEQ ID NO: 66
9.7.2-CG2 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCAIRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfp
epvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssnfgtqtytcnvdhkpsntkvdktverkccvecppcp
appvagpsvflfppkpkdtlmisrtpevtcvvvdvshedpevqfnwyvdgvevhnaktkpreeqfnstfrvvsv
ltvvhqdwlngkeykckvsnkglpapiektisktkgqprepqvytlppsreemtknqvsltclvkgfypsdiave
wesngqpennykttppmldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 70
9.7.2-CG4 Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyfp
epvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcpscp
apeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvvs
vltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdiav
ewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

SEQ ID NO: 86
9.7.2-Ser Heavy Chain [Gamma chain] protein sequence
mefglswvflvaiikgvqcQVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWI
RQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA
EDTAVYYCARRIGGMDVWGQGTTVTVSSAstkgpsvfplapcsrstsestaalgclvkdyf
pepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtktytcnvdhkpsntkvdkrveskygppcppc
papeflggpsvflfppkpkdtlmisrtpevtcvvvdvsqedpevqfnwyvdgvevhnaktkpreeqfnstyrvv
svltvlhqdwlngkeykckvsnkglpssiektiskakgqprepqvytlppsqeemtknqvsltclvkgfypsdia
vewesngqpennykttppvldsdgsfflysrltvdksrwqegnvfscsvmhealhnhytqkslslspgk

Claims

1. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises:

a) a heavy chain comprising the heavy chain CDR1, CDR2 and CDR3 of an antibody selected from the group consisting of: antibodies 8.10.3F and 9.14.4I; or

b) the heavy chain of a) and a light chain comprising the light chain CDR1, CDR2 and CDR3 of the selected antibody.

2. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises:

a) a heavy chain comprising the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of the heavy chain of an antibody selected from the group consisting of: antibodies 8.10.3F and 9.14.4I; or

b) the heavy chain of a) and a light chain comprising the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of the selected antibody.

3. The monoclonal antibody or antigen-binding portion according to claim 1, wherein the antibody or portion comprises any one or more of the FR1, FR2, FR3 and FR4 amino acid sequences of an antibody selected from the group consisting of: antibodies 8.10.3F and 9.14.4I; wherein the CDR and FR amino acid sequences are selected from the same antibody.

4. The monoclonal antibody or antigen-binding portion according to claim 2, wherein the antibody or portion comprises:

a) a heavy chain variable domain (V_H) amino acid sequence of an antibody selected from the group consisting of: antibodies 8.10.3F and 9.14.4I; or

b) the V_H amino acid sequence of a) and a light chain variable domain (V_L) amino acid sequence of the selected antibody.

5. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises the amino acid sequences

of the CDR1, CDR2 and CDR3 found in a light chain comprising SEQ ID NO: 32 and the amino acid sequences of the CDR1, CDR2 and CDR3 found in a heavy chain comprising SEQ ID NO: 30.

6. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises the amino acid sequences of the CDR1, CDR2 and CDR3 found in the heavy chain variable domain of antibody 8.10.3F and the amino acid sequences of the CDR1, CDR2 and CDR3 found in the light chain variable domain of antibody 8.10.3F.

7. A monoclonal antibody that specifically binds to M-CSF, wherein the heavy chain amino acid sequence of said antibody is SEQ ID NO: 30 without the signal sequence, and the light chain amino acid sequence of said antibody is SEQ ID NO: 32 without the signal sequence.

8. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises the amino acid sequences of the CDR1, CDR2 and CDR3 found in a light chain comprising SEQ ID NO: 28 and the amino acid sequences of the CDR1, CDR2 and CDR3 found in a heavy chain comprising SEQ ID NO: 26.

9. A monoclonal antibody or an antigen-binding portion thereof that specifically binds to M-CSF, wherein the antibody or portion comprises the amino acid sequences of the CDR1, CDR2 and CDR3 found in the heavy chain variable domain of antibody 9.14.4I and the amino acid sequences of the CDR1, CDR2 and CDR3 found in the light chain variable domain of antibody 9.14.4I.

10. A monoclonal antibody that specifically binds to M-CSF, wherein the heavy chain amino acid sequence of said antibody is SEQ ID NO: 26 without the signal sequence, and the light chain amino acid sequence of said antibody is SEQ ID NO: 28 without the signal sequence.

11. A human monoclonal antibody 8.10.3F or an antigen-binding portion thereof that specifically binds to M-CSF.

12. A human monoclonal antibody 9.14.4I or an antigen-binding portion thereof that specifically binds to M-CSF.

13. The monoclonal antibody or antigen-binding portion according to any one of claims 1 to 12, wherein the C-terminal lysine of the heavy chain of the antibody or portion is not present.

14. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 30 without the signal sequence.

15. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 26 without the signal sequence.

16. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 28 without the signal sequence.

17. A pharmaceutical composition comprising the monoclonal antibody or antigen-binding portion according to any one of claims 1 to 13, and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition according to claim 17, wherein the monoclonal antibody is monoclonal anti-M-CSF antibody 9.14.4I or 8.10.3F.

19. A monoclonal antibody or antigen-binding portion thereof according to any one of claims 1 to 13 for use in medicine.

20. Use of the monoclonal antibody or antigen-binding portion according to any one of claims 1 to 13, for the preparation of a pharmaceutical composition for treating a condition selected from the group consisting of arthritis, psoriatic arthritis, ankylosing spondylitis, Reiter's syndrome, rheumatoid arthritis, gout, traumatic arthritis, rubella arthritis and acute synovitis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, Alzheimer's disease, stroke, neurotrauma, asthma, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone

resorption disease, osteoporosis, restenosis, cardiac and renal reperfusion injury, thrombosis, glomerularonephritis, diabetes, graft vs. host reaction, allograft rejection, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, muscle degeneration, eczema, contact dermatitis, psoriasis, sunburn, and conjunctivitis shock.

21. Use of the monoclonal antibody or antigen-binding portion according to any one of claims 1 to 13, for the preparation of a pharmaceutical composition for treating rheumatoid arthritis.

22. Use of the monoclonal antibody or antigen-binding portion thereof according to any one of claims 1 to 13, in the preparation of a pharmaceutical composition for treating a solid tumor or a non-solid tumor.

23. The use according to claim 22, wherein the solid tumor is a sarcoma, carcinoma, or lymphoma.

24. The use according to claim 22, wherein the solid tumor is a brain cancer, squamous cell cancer, bladder cancer, gastric cancer, pancreatic cancer, breast cancer, head cancer, neck cancer, liver cancer, esophageal cancer, prostate cancer, colorectal cancer, lung cancer, renal cancer, kidney cancer, ovarian cancer, uterine cancer, gynecological cancer, nasopharyngeal cancer, thyroid cancer, parathyroid cancer, adrenal gland cancer, small intestine cancer, colon cancer, stomach cancer, rectal cancer, anal cancer, skin cancer, head and neck cancer, urethral cancer, penile cancer, melanoma, or a solid tumor of childhood.

25. The use according to claim 22, wherein the non-solid tumor is a lymphoma, leukemia, or multiple myeloma.

26. The use according to claim 22, wherein the non-solid tumor is a cancer of the blood, bone marrow or lymphatic system.

27. An isolated cell line that produces the monoclonal antibody or antigen-binding portion according to any one of claims 1 to 13, or the heavy chain of the antibody or portion.

28. The isolated cell line according to claim 27 that produces a monoclonal antibody selected from the group consisting of: antibodies 8.10.3F, 9.14.4I, and an antibody that has the same amino acid sequence as one of the foregoing antibodies.

29. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the heavy chain, or an antigen-binding portion thereof, or both the heavy chain and light chain, or an antigen-binding portion thereof, of the monoclonal antibody according to any one of claims 1 to 13.

30. A vector comprising the nucleic acid molecule according to claim 29, wherein the vector optionally comprises an expression control sequence operably linked to said nucleic acid molecule.

31. An isolated host cell comprising the vector according to claim 30 or the nucleic acid molecule according to claim 29.

32. The isolated host cell according to claim 31 comprising a nucleic acid molecule encoding the heavy chain and a nucleic acid molecule encoding the light chain of the antibody or antigen-binding portion thereof according to any one of claims 1 to 13.

33. A method of making an anti-M-CSF antibody or antigen-binding portion thereof, comprising culturing the host cell according to claim 31 or the cell line according to claim 27 under suitable conditions and recovering the antibody or portion.