HIGH AFFINITY HUMAN ANTIBODIES TO HUMAN NERVE GROWTH FACTOR

Field of the invention

[0001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind human nerve growth factor (NGF), and therapeutic methods of using those antibodies.

Statement of Related Art

[0002] Nerve growth factor (NGF) was the first neurotrophin to be identified, and its role in the development and survival of both peripheral and central neurons has been well characterized. NGF has been shown to be a critical survival and maintenance factor in the development of peripheral sympathetic and embryonic sensory neurons and of basal forebrain cholinergic neurons (Smeyne et al. (1994) Nature 368:246-249; Crowley et al. (1994) Cell 76:1001-1011). NGF upregulates expression of neuropeptides in sensory neurons (Lindsay et al. (1989) Nature 337:362-364) and its activity is mediated through two different membrane-bound receptors, the TrkA receptor and the p75 common neurotrophin receptor.

[0003] NGF is elevated in synovial fluid in patients suffering from rheumatoid arthritis and other types of arthritis. NGF antagonists have been shown to prevent hyperalgesia and allodynia in animal models of neuropathic and chronic inflammatory pain.

[0004] Anti-NGF antibodies are described in, for example, WO 01/78698, WO 02/096458, WO 2004/032870, US patent publications 2005/0074821, 2004/0237124, and 2004/0219144.

BRIEF SUMMARY OF THE INVENTION

[0005] In a first aspect, the invention provides fully human antibodies and antigen-binding fragments thereof that specifically bind human nerve growth factor (NGF) with a K_D of about 5 pM or less and do not cross-react with neurotrophin-3 (NT-3). In a preferred embodiment, the anti-NGF antibody or fragment thereof binds human NGF with a K_D of 1.0 pM or less. These antibodies are characterized by binding to NGF with high affinity, high specificity and by the ability to neutralize NGF activity. In preferred embodiments, the antibody or fragment thereof binds human NGF about 2-10 fold higher than rat NGF and/or mouse NGF.

[0006] The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to effect functionality, e.g., to eliminate residual effector functions (Glu which eliminates residual effector functions (Reddy et al. (2000) J. Immunol. 164:1925-1933).

[0007] In one embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) selected from the group of SEQ ID NO:4, 20, 36, 52, 68, 84, 100, 104, 108, 112, 116, 132, 136, 140, 156, 160, 176, 180, 184, 200,

204, 208, 224, 228, 232, 236, 240, 256, 260, 264, 280, 284, 288, 304, 308, 312, 328, 332, 336, 352, 356, 360, 376, 380, 384, 400, 404, 420, 424, 440, 456, 460, 464, 480, 484, 488, 504, 508, 512, 528 and 532 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; more preferably, the antibody or fragment thereof comprises the HCVR shown in SEQ ID NO:108, 100 or 84; even more preferably, the HCVR comprises the amino acid sequence shown in SEQ ID NO:108.

[0008] In one embodiment, the antibody or fragment thereof further comprises a light chain variable region (LCVR) selected from the group of SEQ ID NO:12, 28, 44, 60, 76, 92, 102, 106, 110, 114, 124, 134, 138, 148, 158, 168, 178, 182, 192, 202, 206, 216, 226, 230, 234, 238, 248, 258, 262, 272, 282, 286, 296, 306, 310, 320, 330, 334, 344, 354, 358, 368, 378, 382, 392, 402, 412, 422, 432, 448, 458, 462, 472, 482, 486, 496, 506, 510, 520, 530, and 534, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In a preferred embodiment, the LCVR is SEQ ID NO:110, 102 or 92; even more preferably, the LCVR is the amino acid sequence shown in SEQ ID NO:110.

[0009] In specific embodiments, the antibody or fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) sequence pair selected from the group of SEQ ID NO:4/12, 20/28, 36/44, 52/60, 68/76, 84/92, 100/102, 104/106, 108/110, 112/114, 116/124, 132/134, 136/138, 140/148, 156/158, 160/168, 176/178, 180/182, 184/192, 200/202, 204/206, 208/216, 224/226, 228/230, 232/234, 236/238, 240/248, 256/258, 260/262, 264/272, 280/282, 284/286, 288/296, 304/306, 308/310, 312/320, 328/330, 332/334, 336/344, 352/354, 356/358, 360/368, 376/378, 380/382, 384/392, 400/402, 404/412, 420/422, 424/432, 440/448, 456/458, 460/462, 464/472, 480/482, 484/486, 488/496, 504/506, 508/510, 512/520, 528/530 and 532/534. In a preferred embodiment, the HCVR/ LCVR pair is one of SEQ ID NO:108/110, 100/102 or 84/92; more preferably, SEQ ID NO:108/110.

[0010] In a second aspect, the invention features an antibody or antigen-binding fragment of an antibody comprising a heavy chain CDR3 (HCDR3) domain selected from SEQ ID NO:10, 26, 42, 58, 74, 90, 122, 146, 166, 190, 214, 246, 270, 294, 318, 342, 366, 390, 410, 430, 446, 470, 494 and 518, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain selected from 18, 34, 50, 66, 82, 98, 130, 154, 174, 198, 222, 254, 278, 302, 326, 350, 374, 398, 418, 438, 454, 478, 502 and 526, or substantially similar sequences thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In a preferred embodiment, the HCDR3/LCDR3 pair is one of SEQ ID NO:90 and 98; 214 and 222; 410 and 418; 430 and 438; or 446 and 454; more preferably, SEQ ID NO:90 and 98.

[0011] In a further embodiment, the invention comprising an antibody or fragment thereof further comprising a heavy chain CDR1 (HCDR1) domain selected from SEQ ID NO:6, 22, 38, 54, 70, 86, 118, 142, 162, 186, 210, 242, 266, 290, 314, 338, 362, 386, 406, 426, 442, 466, 490, and 514, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least

99% sequence identity; a heavy chain CDR2 (HCDR2) domain selected from SEQ ID NO:8, 24, 40, 56, 72, 88, 120, 144, 164, 188, 212, 244, 268, 292, 316, 340, 364, 388, 408, 428, 444, 468, 492 and 516 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a light chain CDR1 (LCDR1) domain selected from SEQ ID NO:14, 30, 46, 62, 78, 94, 126, 150, 170, 194, 218, 250, 274, 298, 322, 346, 370, 394, 414, 434, 450, 474, 498, and 522, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR2 (LCDR2) domain selected from SEQ ID NO:16, 32, 48, 64, 80, 96, 128, 152, 172, 196, 220, 252, 276, 300, 324, 348, 372, 396, 416, 436, 452, 476, 500 and 524, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In a preferred embodiment, the heavy and light chain CDR sequences SEQ ID NO:86, 88, 90, 94, 96 and 98; 210, 212, 214, 218, 220 and 222; 406, 408, 410, 414, 416 and 418; 442, 444, 446, 450, 452 and 454; and 426, 428, 430, 434, 436 and 438; even more preferably, the CDR sequences are SEQ ID NO:86, 88, 90, 94, 96 and 98.

[0012] In a third aspect, the invention provides nucleic acid molecules encoding anti-NGF antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0013] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from SEQ ID NO:3, 19, 35, 51, 67, 83, 99, 103, 107, 111, 115, 131, 135, 139, 155, 159, 175, 179, 183, 199, 203, 207, 223, 227, 231, 235, 239, 255, 259, 263, 279, 283, 287, 303, 307, 311, 327, 331, 335, 351, 355, 359, 375, 379, 383, 399, 403, 419, 423, 439, 455, 459, 463, 479, 483, 487, 503, 507, 511, 527 and 531, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; more preferably, the HCVR is encoded by SEQ ID NO:107 or 99.

[0014] In one embodiment, the antibody or fragment thereof further comprises a LCVR encoded by a nucleic acid sequence selected from SEQ ID NO:11, 27, 43, 59, 75, 91, 101, 105, 109, 113, 123, 133, 137, 147, 157, 167, 177, 181, 191, 201, 205, 215, 225, 229, 233, 237, 247, 257, 261, 271, 281, 285, 295, 305, 309, 319, 329, 333, 343, 353, 357, 367, 377, 381, 391, 401, 411, 421, 431, 447, 457, 461, 471, 481, 485, 495, 505, 509, 519, 529 and 533, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; preferably, the LCVR is encoded by SEQ ID NO:109 or 101.

[0015] In one embodiment, the invention features an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from SEQ ID NO:9, 25, 41, 57, 73, 89, 121, 145, 165, 189, 213, 245, 269, 293, 317, 341, 365, 389, 409, 429, 445, 469, 493 and 517, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR3 domain encoded by a nucleotide

sequence selected from SEQ ID NO: 17, 33, 49, 65, 81, 97, 129, 153, 173, 197, 221, 253, 277, 301, 325, 349, 373, 397, 417, 437, 453, 477, 501 and 525, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In a preferred embodiment, the HCDR3 and LCDR3 sequences are encoded by SEQ ID NO:89 and 97, respectively.

[0016] In a further embodiment, the antibody or fragment thereof further comprises, a HCDR1 domain encoded by a nucleotide sequence selected from SEQ ID NO:5, 21, 37, 53, 69, 85, 117, 141, 161, 185, 209, 241, 265, 289, 313, 337, 361, 385, 405, 425, 441, 465, 489 and 513, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a HCDR2 domain encoded by a nucleotide sequence selected from SEQ ID NO:7, 23, 39, 55, 71, 87, 119, 143, 163, 187, 211, 243, 267, 291, 315, 339, 363, 387, 407, 427, 443, 467, 491 and 515, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; a LCDR1 domain encoded by a nucleotide sequence selected from SEQ ID NO:13, 29, 45, 61, 77, 93, 125, 149, 169, 193, 217, 249, 273, 297, 321, 345, 369, 393, 413, 433, 449, 473, 497 and 521, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR2 domain encoded by a nucleotide sequence selected from SEQ ID NO:15, 31, 47, 63, 79, 95, 127, 151, 171, 195, 219, 251, 275, 299, 323, 347, 371, 395, 415, 435, 451, 475, 499 and 523, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In a preferred embodiment, the antibody or fragment thereof comprises heavy chain CDR sequences encoded by SEQ ID NO:85, 87, and 89; and light chain CDR sequences encoded by SEQ ID NO:93, 95 and 97.

[0017] In a fourth aspect, the invention features an isolated antibody or antigen-binding fragment of an antibody that specifically binds human NGF, comprising a HCDR3 and a LCDR3, wherein the HCDR3 comprises an amino acid sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11}$ - $X^{12} – X^{13} – X^{14} – X^{15} – X^{16} – X^{17} – X^{18}$ (SEQ ID NO:537) wherein X^1 is Ala or Ser, X^2 is Thr or Lys, X^3 is Glu or Ile, X^4 is Phe or Gly, X^5 is Val or Gly, X^6 is Val or Trp, X^7 is Val or Phe, X^8 is Thr or Gly, X^9 is Asn or Lys, X^{10} is Phe or Leu, X^{11} is Asp or Phe, X^{12} is Asn or Ser, X^{13} is Ser or absent, X^{14} is Tyr or absent, X^{15} is Gly or absent, X^{16} is Met or absent, X^{17} is Asp or absent, and X^{18} is Val or absent; and the LCDR3 comprises an amino acid sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9$ (SEQ ID NO:540) wherein X^1 is Gln, X^2 is Gln, X^3 is Tyr, X^4 is Asn, X^5 is Arg or Asn, X^6 is Tyr or Trp, X^7 is Pro, X^8 is Tyr or Trp, and X^9 is Thr.

[0018] In a more specific embodiment, the antibody or fragment thereof further comprise a HCDR1 sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO:535), wherein X^1 is Gly, X^2 is Phe, X^3 is Thr or Asn, X^4 is Phe or Leu, X^5 is Thr or Asp, X^6 is Asp or Glu, X^7 is Tyr or Leu, and X^8 is Ser or Ala; a HCDR2 sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8$ (SEQ ID NO:536), wherein X^1 is Ile or Phe, X^2 is Asp or Ser, X^3 is Pro or Trp, X^4 is Glu or Asn, X^5 is Asp or

Ser, X^6 is Gly, X^7 is Thr, Glu or Ser, X^8 is Thr or Ile; a HCDR3 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18}$ (SEQ ID NO:537) wherein X^1 is Ala or Ser, X^2 is Thr or Lys, X^3 is Glu or Ile, X^4 is Phe or Gly, X^5 is Val or Gly, X^6 is Val or Trp, X^7 is Val or Phe, X^8 is Thr or Gly, X^9 is Asn or Lys, X^{10} is Phe or Leu, X^{11} is Asp or Phe, X^{12} is Asn or Ser, X^{13} is Ser or absent, X^{14} is Tyr or absent, X^{15} is Gly or absent, X^{16} is Met or absent, X^{17} is Asp or absent, and X^{18} is Val or absent; a LCDR1 sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6$ (SEQ ID NO:538) wherein X^1 is Gln or Arg, X^2 is Ala, Ser or Thr, X^3 is Val or Ile, X^4 is Arg or Thr, X^5 is Asn, Phe or Tyr, and X^6 is Asp or Asn; a LCDR2 sequence of the formula $X^1 - X^2 - X^3$ (SEQ ID NO:539) wherein X^1 is Gly or Ala, X^2 is Ala, and X^3 is Ser or Phe; and a LCDR3 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9$ (SEQ ID NO:540) wherein X^1 is Gln, X^2 is Gln, X^3 is Tyr, X^4 is Asn, X^5 is Arg or Asn, X^6 is Tyr or Trp, X^7 is Pro, X^8 is Tyr or Trp, and X^9 is Thr.

[0019] In a fifth aspect, the invention features a fully human antibody or antibody fragment which blocks NGF activity with an IC_{50} of less than about 10 nM, as measured in *in vitro* PC12 cell-based assay (described below). In a preferred embodiment, the antibody of the invention exhibits an IC_{50} of about 500 pM or less; even more preferably, an IC_{50} of about 100 pM or less; about 50 pM or less; or about 25 pM or less.

[0020] In one embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that binds NGF with a K_D of less than about 500 pM, preferably less than about 300 pM, even more preferably less than about 100 pM, less than about 50 pM, less than about 20 pM; less than about 10 pM, less than about 5 pM, or less than about 1 pM, as determined by surface plasmon resonance (BIACORE™). In a preferred embodiment, the anti-NGF human antibody or antibody fragment of the invention binds human NGF with a K_D of about 0.5 pM or less. In preferred embodiments, the antibody or fragment thereof binds human NGF about 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold higher affinity than rat NGF and about 1.5-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or 10-fold higher than mouse NGF.

[0021] In a preferred embodiment, the antibody or fragment thereof exhibits high specificity for human NGF, for example, does not cross-react with closely related neurotrophin-3 (NT-3). Thus, in a preferred embodiment, the high affinity and high selectivity antibody or fragment thereof exhibits a K_D for human NGF of 1.0 pM or less, inhibits binding of NGF to receptors TrkA and p75, and does not cross-react with human NT-3, as measured by surface plasmon resonance. NT-3 plays a critical role in such physiological processes as, for example, muscle motor neuron coordination, and thus, antibodies or antibody fragments that do not cross-react with NT-3 provide an unexpected clinical and therapeutic advantage over prior art antibodies. NT-3 has been shown to prevent the development of thermal hyperalgesia in the CCI model of neuropathic pain (see, for example, Wilson-Gerwing et al. (2005) J Neuroscience 25:758-767). More recently, exogenous NT-3 has

been shown to significantly decrease expression of two sodium channels which appear to play a role in the generation of neuropathic pain (Wilson-Gerwing & Verge (2006) Neuroscience 141:2075-2085. These data suggest a beneficial role of NT-3 in neuropathic pain.

[0022] The invention encompasses anti-NGF antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0023] In a sixth aspect, the invention features a composition comprising a recombinant human antibody or fragment thereof that specifically binds NGF and an acceptable carrier. In a related aspect, the invention features a composition that is a combination of an NGF inhibitor and a second therapeutic agent. In one embodiment, the NGF inhibitor is an antibody or fragment thereof. In a preferred embodiment, the second therapeutic agent is any suitable therapeutic agent that is advantageously combined with an NGF inhibitor.

[0024] In a seventh aspect, the invention features methods for inhibiting human NGF activity using the anti-NGF antibody or antigen-binding portion of the antibody of the invention. The disorder treated is any disease or condition that is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of NGF activity. More specifically, the invention provides a method of treating an NGF-mediated condition or disease such as inflammatory pain, post-operative incision pain, complex regional pain syndrome, primary or metastatic bone cancer pain, neuropathic pain, fracture pain, osteoporotic fracture pain, pain resulting from burn, osteoporosis, gout joint pain, pains associated with sickle cell crises, and other nociceptic pains, as well as hepatocellular carcinoma, breast cancer, and liver cirrhosis, by administering an NGF inhibitor, such as the antibody or antibody fragment of the invention, as a single agent, or with a second therapeutic agent. In preferred embodiments of neuropathic pain, referred trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, reflex sympathetic dystrophy and neurogenic pain conditions are preferably treated. The second therapeutic agent may be an interleukin-1 (IL-1) inhibitor, for example, a fusion protein such as that described in U.S. 6,927,044; or an antiepileptic drug, such as gabapentain, pregabalin, topiramate; or a tricyclic antidepressant, such as amitriptyline; celecoxib; a cytokine antagonist, such as an antagonist protein or antibody against IL-1, IL-6, IL-6R, IL-18 or IL-18R. In one embodiment, the second therapeutic agent is another neurotrophin, for example, NT-3.

[0025] In an eighth aspect, the invention provides an antibody or antigen-binding fragment as described above for use to attenuate or inhibit a NGF-mediated disease or condition in a human. The NGF-mediated condition or disease is inhibited without significant impairment of motor coordination, and is one of inflammatory pain, post-operative incision pain, neuropathic pain, fracture pain, gout joint pain, post-herpetic neuralgia, pain resulting from burns, cancer pain,

osteoarthritis or rheumatoid arthritis pain, sciatica, pain associated with sickle cell crises, or post-herpetic neuralgia.

[0026] In a related aspect, the invention provides use of an antibody or antigen-binding fragment of an antibody as described above in the manufacture of a medicament for use to attenuate or inhibit an NGF-mediated disease or condition in a human.

[0027] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0028] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0029] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0030] The term “human nerve growth factor” or "NGF", as used herein, refers to human NGF having the nucleic acid sequence shown in SEQ ID NO:1 and the amino acid sequence of SEQ ID NO:2, or a biologically active fragment thereof.

[0031] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0032] The term "antigen-binding portion" of an antibody (or simply "antibody portion" or “antibody fragment”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., NGF). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding

fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) Nature 241:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL 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 et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448; Poljak et al. (1994) Structure 2:1121-1123).

[0033] Still further, an antibody or antigen-binding portion thereof may be part of larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al. (1995) Human Antibodies and Hybridomas 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al. (1994) Mol. Immunol. 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

[0034] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0035] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0036] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds NGF is substantially free of antibodies that specifically bind antigens other than NGF). An isolated antibody that specifically binds NGF may, however, have cross-reactivity to other antigens, such as NGF molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0037] A "neutralizing antibody", as used herein (or an "antibody that neutralizes NGF activity"), is intended to refer to an antibody whose binding to NGF results in inhibition of the biological activity of NGF. This inhibition of the biological activity of NGF can be assessed by measuring one or more indicators of NGF biological activity, such as NGF-induced cellular activation and NGF binding to NGF receptor. These indicators of NGF biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see examples below).

[0038] A “CDR” or complementarity determining region is a region of hypervariability interspersed with regions that are more conserved, termed “framework regions”. A group of CDRs may be defined as an amino acid consensus sequence.

[0039] 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.).

[0040] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0041] The term "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind NGF is intended to refer to

a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than NGF, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a VH region of an anti-NGF antibody contains no other sequences encoding other VH regions that bind antigens other than human NGF.

[0042] The term "epitope" includes any determinant, preferably a polypeptide determinant, capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In preferred embodiments, an antibody is said to specifically bind an antigen when the equilibrium dissociation constant is less than or equal to 10^{-8} M, more preferably when the equilibrium dissociation constant is less than or equal to 10^{-9} M, and most preferably when the dissociation constant is less than or equal to 10^{-10} M.

[0043] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates 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 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 below.

[0044] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions which 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 or degree of similarity 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 (1994) Methods Mol. Biol. 24: 307-331. 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: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0045] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters 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 with default or recommended parameters; a program in GCG Version 6.1. 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 (2000) *supra*). 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. See, e.g., Altschul et al. (1990) J. Mol. Biol. 215: 403 410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389 402.

Preparation of Human Antibodies

[0046] Methods for generating human antibodies include, for example, VELOCIMMUNE™, XENOMOUSE™ technology (Green et al. (1994) Nature Genetics 7:13-21), the “minilocus” approach, and phage display. The VELOCIMMUNE™ technology (US 6,596,541, Regeneron Pharmaceuticals) encompasses a method of generating a high specificity fully human antibody to a select antigen. This technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody. In specific embodiment, the cell is a CHO cell.

[0047] Antibodies may be therapeutically useful in blocking a ligand-receptor interaction or inhibiting

receptor component interaction, rather than by killing cells through fixation of complement and participation in complement-dependent cytotoxicity (CDC), or killing cells through antibody-dependent cell-mediated cytotoxicity (ADCC). The constant region of an antibody is thus important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0048] Human immunoglobulins can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0049] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, CH2 or CH3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0050] Antibodies of the invention are preferably prepared with the use of VELOCIMMUNE™ technology. A transgenic mouse in which the endogenous immunoglobulin heavy and light chain variable regions are replaced with the corresponding human variable regions is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0051] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-9} through about 10^{-12} M or higher, for example, at least about 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M or at least 10^{-12} M, when measured by binding to antigen either immobilized on solid phase or in solution phase.

[0052] Initially, high affinity chimeric antibodies are isolated having a human variable region and a

mouse constant region. As described below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4 (for example, SEQ ID NO:541, 542 or 543). While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Epitope Mapping and Related Technologies

[0053] To screen for antibodies that bind to a particular epitope (e.g., those which block binding of IgE to its high affinity receptor), a routine cross-blocking assay such as that described in Harlow and Lane (1990) *supra* can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496).

[0054] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0055] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the anti-NGF antibodies of the invention into groups of antibodies binding different epitopes.

Immunoconjugates

[0056] The invention encompasses a human anti-NGF monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxin agents include any agent that is detrimental to cells. Examples of suitable cytotoxin agents and chemotherapeutic agents for forming immunoconjugates are known in the art, see for example, WO 05/103081).

Bispecifics

[0057] The antibodies of the present invention may be monospecific, bispecific, or multispecific. Multispecific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al. (1991) J. Immunol. 147:60-69. The human anti-NGF antibodies can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment, to produce a bispecific or a multispecific antibody with a second binding specificity.

Therapeutic Administration and Formulations

[0058] The invention provides therapeutic compositions comprising the anti-NGF antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0059] The dose may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present invention is used for treating various conditions and diseases associated with NGF, including inflammatory pain, neuropathic and/or nociceptive pain, hepatocellular carcinoma, breast cancer, liver cirrhosis, and the like, in an adult patient, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. preferably about 0.1 to about 10 mg/kg body weight, and more preferably about 0.1 to about 5 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted.

[0060] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see,

e.g., Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0061] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see Langer (1990) Science 249:1527-1533; Treat et al. (1989) in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.

[0062] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton (1987) CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974). In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138, 1984). Other controlled release systems are discussed in the review by Langer (1990) Science 249:1527-1533.

[0063] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0064] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the antibody contained is generally about 5 to 500 mg per dosage form in a unit dose; in the form of injection, it is preferred that the antibody is contained in about 5 to 100 mg and in about 10 to 250 mg for the other dosage forms.

[0065] Single and combination therapies. The invention provides therapeutic methods in which the antibody or antibody fragment of the invention is useful to treat pain associated with a variety of conditions involving NGF. The anti-NGF antibodies or antibody fragments of the invention are particularly useful for the treatment of pain resulting from any condition associated with neurogenic, neuropathic or nociceptic pain. In preferred embodiments of neuropathic pain, referred trigeminal neuralgia, post-herpetic neuralgia, phantom limb pain, fibromyalgia, reflex sympathetic dystrophy and neurogenic pain conditions are preferably treated. In other preferred embodiments, cancer pain, particularly, bone cancer pain, osteoarthritis or rheumatoid arthritis pain, lower back pain, post-operative incision pain, fracture pain, osteoporotic fracture pain, osteoporosis, gout joint pain, diabetic neuropathy, sciatica, pains associated with sickle cell crises, migraine, and other neuropathic and/or nociceptic pain are preferably treated.

[0066] Other indications include, for example, treatment for breast cancer (Adriaenssens et al. (2008) Cancer Res 68:346-51). In specific embodiments of the therapeutic methods of the invention, a subject suffering from joint pain associated with gout is treated with a combination of an antibody or antibody fragment of the invention and optionally with a second therapeutic agent. In one embodiment, the second therapeutic agent is preferably an interleukin-1 (IL-1) antagonist such as rilonacept ("IL-1 trap"; Regeneron). Suitable second therapeutic agents may be one or more agents selected from the group consisting of rilonacept, anakinra (KINERET®, Amgen), a recombinant, nonglycosylated form of the human IL-1 receptor antagonist (IL1Ra), an anti-IL-18 drug such as IL-18BP or a derivative, an IL-18 Trap, or an antibody such as an anti-IL-18, anti-IL-18R1, anti-IL-18Racp, anti-IL-6 and/or anti-IL6Ra antibody. Other co-therapies which may be combined with an NGF antibody or antigen-binding fragment thereof, alone or in combination with an IL-1 antagonist, include low dose colchine, aspirin or other NSAIDs, steroids such as prednisolone, methotrexate, low dose cyclosporine A, TNF inhibitors such as ENBREL®, or HUMIRA®, other inflammatory inhibitors such as inhibitors of caspase-1, p38, IKK1/2, CTLA-4Ig, etc., and/or co- therapies such as uric acid synthesis inhibitors to inhibit the accumulation of uric acid in the body, for example, allopurinol, uric acid excretion promoters to accelerate the rapid excretion of uric acid accumulated in the body, for example, probenecid, sulfinpyrazone and/or benzbromarone are examples of uric acid excretion promoters; corticosteroids; non-steroidal anti-inflammatory drugs (NSAIDs), anti-epileptic drugs such as topiramate; gabapentin, pregabablin; celecoxib; or another neurotrophin, such as NT-3.

EXAMPLES

[0067] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts,

temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric. The statistical analyses were conducted according to mixed Factorial ANOVA with Bondferroni post hoc or Turkey HSD post hoc tests.

Example 1. Immunization and Antibody Generation

[0068] Immunization of rodents can be done by any methods known in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual: Cold Spring Harbor Press, New York; Malik and Lillehoj, Antibody techniques: Academic Press, San Diego). In a preferred embodiment, human NGF protein is administered directly to mice which have DNA loci encoding both human Ig heavy chain variable region and Kappa light chain variable region (VELOCIMMUNE™, Regeneron; US 6,596,541), with an adjuvant to stimulate the immune response. Such an adjuvant includes complete and incomplete Freund's adjuvant, MPL+TDM adjuvant system (Sigma), or RIBI (muramyl dipeptides) (see O'Hagan, Vaccine Adjuvant, Human Press, 2000, Totawa, NJ). Such an adjuvant can prevent rapid dispersal of polypeptide by sequestering the antigen in a local depot, and may contain factors that can stimulate host immune response. In one embodiment, NGF is administered indirectly as a DNA plasmid that contains NGF gene and expresses NGF using the host cellular protein expression machinery to produce antigen polypeptide *in vivo*. In both approaches, to obtain optimal anti-antigen responses, mice are given boost injections every 3~4 weeks and serum samples are collected 10 days after each injection. The antibody immune response is monitored using standard antigen direct binding ELISA methods. Post-boost serum samples diluted in 3-fold serial dilutions are applied to NGF coated plates. Serum titer is defined as the dilution of serum sample that yielded two-fold over background signal in the assay. Animals with optimal responses receive a final boost via intravenous and intra-peritoneal injections without an adjuvant 3~4 days prior to sacrifice. The harvested splenocytes are processed as described below in order to obtain antigen specific monoclonal antibodies.

Example 2. Monoclonal Antibody Isolation

[0069] In one embodiment, antibody-expressing B cells are fused with mouse myeloma cells to form hybridoma cells. The hybrid cells are plated in 96-well plates under HAT selection and allowed to grow for 10 to 20 days. The conditioned media from wells with growing hybridoma cells is screened for antigen binding and receptor blocking activities as described below. Hybridoma cells expressing antibodies of interest are single-cell sub-cloned using flow cytometry, and VH and VL genes from clonal hybridoma cells cloned and sequenced. Antibody proteins are also purified from cultures of antigen specific hybridomas using IgG depleted medium (Invitrogen) and characterized as described below.

[0070] In another embodiment, antigen specific antibodies are isolated directly from antigen positive B cells without being immortalized with specific myeloma cells, and a host CHO cell producing a stable recombinant antibody is generated, as described in USSN 11/809,482 (US patent Publication No. 2007/0280945).

Example 3. Primary Antigen Binding and Receptor Blocking Screening

[0071] To identify antigen specific antibody producing hybridomas, conditioned media was sampled from 96-well culture plates 10 to 20 days after fusion, and antigen binding specificity determined using high through-put direct binding ELISA. Briefly, the condition media at 1:10 and 1:100 fold dilution were allowed to bind to recombinant NGF protein coated MAXISORB™ plates (Nunc) at 100 ng/well. The plate-bound antibodies were detected using goat anti-mouse IgG Fcγ specific HRP conjugated polyclonal antibody (Jackson Immuno Lab). Plates were developed using TMB substrates (BD Pharmigen) and optical density at OD_{450nm} recorded. In parallel, samples at the same dilutions were applied to a streptavidin presented biotin-labeled NGF plates, and the plate bound antibodies detected. Wells showing binding activity to either plate were selected for cell culture expansion and cryo-preserved, and antibody containing supernatants were used for further analysis to obtain specificity, affinity, and functionality profile.

[0072] In addition to the direct antigen binding screening, functional screening was also utilized in order to identify clones secreting antibody with desirable properties. Maxisorb plates were coated with 100 ng/well recombinant human TrkA-hFc overnight at 4°C. Conditioned media at 1:2 and 1:10 fold dilutions were allowed to bind to 2 ng/ml biotin-NGF in solution for 1 hour prior to transfer to the TrkA-hFc coated plates for the measurement of plate-bound biotin-NGF. The plate-bound biotin-NGF was detected using HRP conjugated streptavidin (Pierce) and developed using TMB substrates (BD Pharmingen) and optical density recorded. Hybridomas in which culture media prevented binding of biotin-NGF to TrkA-hFc were identified as potential blockers and were further characterized.

[0073] Similar *in vitro* screens were applied to 96-well conditioned medium from CHO cells transfected with the fully human IgG containing V genes isolated directly from antigen positive B cells. In addition, samples were screened for NGF binding activity using antigen-coated LUMINEX™ beads, to which antigen specific bound antibody was detected using PE-conjugated goat anti-human IgG Fcγ-specific polyclonal antibodies. The antigen-binding antibodies were subjected to affinity measurement using BIACORE™. Briefly, antibodies from crude culture supernatants were captured on an amine coupled hFc specific polyclonal antibody surface. Antigen binding at a single concentration was monitored. A 1:1 bimolecular interaction model was used to fit the binding sensogram to determine the antigen-binding affinities (K_D) using the kinetic rate constants ka and kd for each antibody interaction under identical conditions. Specifically, goat anti-human IgG Fcγ-specific polyclonal antibodies were covalently coupled onto CM-5 chip surfaces,

and antibody-containing CHO supernatants were injected at 1 μl/min for 5 minutes followed by a buffer wash. Human NGF (25 nM) was injected for 3 minutes to allow NGF to bind to the human antibody immobilized surface. Immediately following NGF injection, the surfaces were buffer injected at 100 μl/min for ~ 10 minutes and the decay of RU signal recorded. Surfaces were regenerated to remove bound antibody and NGF, and the cycle repeated with the next CHO supernatant sample.

Example 4. Antigen Binding Affinity Determination

[0074] Antigen binding affinities of the antibodies for human NGF were determined by surface kinetics using a real-time biosensor surface plasmon resonance assay (BIACORE™). Antibodies were captured on either a goat, anti-human or anti-mouse IgG polyclonal antibody surface created by direct amine coupling of the capture antibody to a BIACORE™ chip. Various concentrations of human NGF were injected over the captured antibody surfaces while the association of the antigen to the antibody and the dissociation of the bound complex were monitored in real time. Kinetic analysis was performed to obtain the equilibrium dissociation constant (K_D) and dissociation rate constant, and the latter was used to calculate the antigen/antibody complex dissociation $t_{1/2}$ (Table 1). A humanized anti-human NGF monoclonal antibody E3 ("RN624") (tanezumab; CAS Registry No. 880266-57-9; US patent publication 2004/0237124) was used as the control.

Table 1

Antibody	K_D (pM)	$t_{1/2}$
301272-1D07-B10	0.5	34.6 hr
301272-1H07-G9	60.1	32.8 min
301272-1H08-G8	0.2	55.6 hr
301272-3D08-C11	0.7	6.9 hr
301272-3F12-D7	190.0	13.2 min
301272-3G11-C1	1.1	14.6 hr
301272-3H10-A10	0.1	25.2 hr
301272-3H11-A3	23.8	4.3 hr
301272-6E07-D10	13.0	4.5 hr
301272-6G10-D7	7.7	44.3 min
301272-7A10-D7	75.0	11.6 min
301272-7C05-G1	162.0	10.1 min
301272-7E05-F6	0.4	40.0 hr
301272-7F11-A8	5.8	5.3 hr
301272-7G09-E4	17.0	4.3 hr
301272-7G10-E1	292.0	10.1 min

301272-7G11-F6	4.9	2.9 hr
301272-7H05-D4	77.6	1.0 hr
301272-7H07-C12	9.8	6.0 hr
VAT 8C10-8	102.0	14.7 min
VAT 13F5-5	156.0	13.7 min
VAT 12A10-13	109.0	9.4 min
VAT 2C2-1	959.0	9.0 min
Control (RN624)	1.3	35.0 hr

[0075] Antigen binding affinities of selected purified antibodies for NGF were also determined by surface kinetics employing a real-time biosensor surface plasmon resonance assay (BIACORE™) as described above. For convenience, antibody 301272-3H10-A10 was renamed "REGN261" (HCVR/LCVR SEQ ID NOs:84/92 and hIgG1 SEQ ID NO:541); 301272-6E07-D10 was renamed "REGN263" (HCVR/LCVR SEQ ID NO:208/216 and hIgG1 SEQ ID NO:541). Derived antibodies tested included REGN472 (HCVR/LCVR SEQ ID NO:100/102 and hIgG1 SEQ ID NO:541), REGN474 (HCVR/LCVR SEQ ID NO:100/102 and mutant hIgG4 SEQ ID NO:543), REGN475 (HCVR/LCVR SEQ ID NO:108/110 and mutant hIgG4 SEQ ID NO:543), REGN476 (HCVR/LCVR SEQ ID NO:224/226 and mutant hIgG4 SEQ ID NO:543), and REGN477 (HCVR/LCVR SEQ ID NO:232/234 and mutant hIgG4 SEQ ID NO:543).

Table 2

Antibody	K_D (pM)	$t_{1/2}$ (hr)
REGN472	0.41	30
REGN474	0.41	31
REGN475	0.18	57
REGN476	8.91	4
REGN477	7.98	4
Control (RN624)	1.25	35

Example 5. Cross-reactivity to Neurotrophin-3 (NT-3)

[0076] NGF and NT-3 belong to nerve growth factor family and are small, basic, secretory proteins that allow the survival of specific neuronal populations. Though these two neurotrophins share some amino acid identities, biological functions may vary (Barde et al. 1990 Prog Growth Factor Res 2(4):237-48).

[0077] The anti-NGF antibodies were examined for binding cross-reactivity with human NT-3. Briefly, goat anti-human IgG polyclonal antibody was chemically linked to a CM5 chip. Anti-NGF monoclonal antibodies were injected forming a surface of about 50 to 900 RU of immobilized

antibody through the interaction with the chip coupled polyclonal antibodies. NGF or NT-3 protein at a concentration of 20 nM was injected over the surface, followed by a buffer wash to allow bound ligand to dissociate. Both association and dissociation phases were monitored and data were analyzed. Results are shown in Table 3 (NB = no binding activity observed). In contrast to the control antibody (RN624), all of the test antibodies showed no measurable binding to NT-3, thus indicating a higher degree of antigen specificity relative to the control antibody.

Table 3

Antibody	NGF K_D (pM)	NT-3 K_D (nM)
301272-1D07-B10	0.5	NB
301272-1H07-G9	60.1	NB
301272-1H08-G8	0.2	NB
301272-3D08-C11	0.7	NB
301272-3F12-D7	190.0	NB
301272-3G11-C1	1.1	NB
301272-3H10-A10	0.1	NB
301272-3H11-A3	23.8	NB
301272-6E07-D10	4.3	NB
301272-6G10-D7	7.7	NB
301272-7A10-D7	75.0	NB
301272-7C05-G1	162.0	NB
301272-7E05-F6	0.1	NB
301272-7F11-A8	7.5	NB
301272-7G09-E4	5.5	NB
301272-7G10-E1	292.0	NB
301272-7G11-F6	4.9	NB
301272-7H05-D4	77.6	NB
301272-7H07-C12	9.8	NB
Control (RN624)	1.3	1.1

[0078] OCTET™-based solution competition assays were also employed to measure the ability of REGN475 and RN624 to compete in solution for the binding to NT-3, NGF or human brain derived neurotrophic factor (hBDNF). Briefly, antibody-antigen samples were prepared by pre-incubating control antibody RN624 (2.5 μg/ml) or REGN475 (2.5 μg/ml), with various concentrations of NT-3 (0 to 4 μM), hBDNF (0 to 4 μM) or NGF (0 to 0.2 μM) for 1 hour at 30°C. Streptavidin high binding FA sensors (HBS, ForteBio, Inc., CA) were incubated with biotin-NGF at 2 μg/ml for 10 min at 30°C. Biotin-NGF bound sensors were then incubated with the pre-incubated antigen-antibody samples

for 10 min at 30°C. Changes in the thickness of the biological layer were measured after incubation. The binding was normalized as a percentage of binding relative to the binding of antibody in absence of competitor. As shown in Table 4, the binding between NGF and RN624 was blocked by NT-3 in a dose-dependent manner, whereas binding between REGN475 and NGF was not blocked by NT-3. The presence of hBDNF did not block the binding of either RN624 or REGN475 to NGF, whereas the presence of soluble NGF almost completely blocked the binding of both RN624 and REGN475 to immobilized NGF.

Table 4

Competitor	RN624 % Binding	REGN475 % Binding
NT-3 (4 μM)	17	102
NT-3 (2 μM)	26	102
NT-3 (1 μM)	38	98
NT-3 (0.5 μM)	52	93
NT-3 (0.25 μM)	72	101
NT-3 (0 μM)	100	100
BDNF (4 μM)	103	116
BDNF (2 μM)	104	115
BDNF (1 μM)	104	106
BDNF (0 μM)	100	100
NGF(0.2 μM)	1	3
NGF(0.1 μM)	-1	2
NGF(0.05 μM)	0	1
NGF(0 μM)	100	100

[0079] The binding between selected purified human anti-NGF antibody REGN472, REGN474, REGN475, REGN476, REGN477, or control antibody RN624 and NT-3 was also evaluated using the BIACORE™ assay with NT-3 concentrations ranging from 1.25 nM to 40 nM. While control antibody (RN624) bound NT-3 with a K_D of 1.1 nM, none of the test antibodies exhibited measurable affinity for NT-3.

Example 6. Cross-reactivity to Murine and Rat NGF

[0080] Human NGF (NGF) is highly homologous in amino acid sequence to mouse NGF (mNGF) and rat NGF (rNGF) with about 90% identity. The binding affinities of the antibodies to both mNGF and rNGF were determined as described above. All antibodies showed cross-reactivity to both mNGF and rNGF. One group of antibodies bound NGF from all species strongly with a K_D value of

less than 10 pM; a second group preferably bound NGF and exhibited K_Ds > ~ 100 pM for mNGF and rNGF (control = RN624) (Table 5).

Table 5

Antibody	Human NGF K_D (pM)	mNGF K_D (pM)	rNGF K_D (pM)
301272-1D07-B10	0.5	3.0	6.6
301272-1H07-G9	60.1	2280.0	6330.0
301272-1H08-G8	0.2	1.7	0.7
301272-3D08-C11	0.7	5.0	8.5
301272-3F12-D7	190.0	3130.0	8710.0
301272-3G11-C1	1.1	6.1	5.9
301272-3H10-A10	0.1	0.2	0.6
301272-3H11-A3	23.8	619.0	800.0
301272-6E07-D10	13.0	362.0	360.0
301272-6G10-D7	7.7	94.7	157.0
301272-7A10-D7	75.0	2630.0	4900.0
301272-7C05-G1	162.0	2000.0	1790.0
301272-7E05-F6	0.4	4.1	1.6
301272-7F11-A8	5.8	320.0	459.0
301272-7G09-E4	16.8	379.0	425.0
301272-7G10-E1	292.0	7090.0	11800.0
301272-7G11-F6	4.9	157.0	160.0
301272-7H05-D4	77.6	5520.0	7090.0
301272-7H07-C12	9.8	1200.0	473.0
Control (RN624)	1.25	1.4	1.5

[0081] The binding affinity of selected purified anti-NGF antibodies to mNGF and rNGF were also determined (Table 6).

Table 6

Antibody	NGF K_D (pM)	mNGF K_D (pM)	rNGF K_D (pM)
REGN472	0.41	0.61	3.96
REGN474	0.41	0.43	3.42
REGN475	0.18	0.36	0.93
REGN476	8.91	115	155
REGN477	7.98	133	164
Control (RN624)	1.25	1.4	1.51

Example 7. Inhibition of NGF Binding to Receptors TrkA/hFc and p75/hFc

[0082] To identify blocking antibodies, a receptor blocking assay was designed using a BIACORE™ 3000 instrument. Recombinant human TrkA-hFc and human p75-hFc proteins were amine-coupled to a CM5 chip to a density of about 5000 – 6000 RU. Human NGF (10 nM to 25 nM) was bound to the TrkA and p75 surface to determine maximal RU for NGF binding. The surface was then regenerated and 10 nM to 25 nM NGF was mixed with excess molar concentrations of the individual antibodies or soluble receptorbodies, and the solution was injected over the regenerated chip surface to determine the remaining free NGF binding signals. Table 7 shows the percentage free NGF bound to TrkA and p75 in the presence of antibody or receptorbody. The maximal RU binding of human NGF in the absence of antibody was given a relative value of 100%. As positive controls, RN624, TrkA-hFc and p75-hFc in solution were used and, as a negative binding control, IgG1 control (AVASTIN®; Genentech, CA) was used.

Table 7

Antibody	% Binding TrkA-hFc	% Binding p75-hFc
NGF alone	100	100
301272-1D07-B10	2	4
301272-1H07-G9	20	25
301272-1H08-G8	1	3
301272-3D08-C11	1	2
301272-3F12-D7	25	23
301272-3G11-C1	1	1
301272-3H10-A10	1	2
301272-3H11-A3	18	20
301272-6E07-D10	4	6
301272-6G10-D7	2	2
301272-7A10-D7	31	26
301272-7C05-G1	1	1
301272-7E05-F6	1	1
301272-7F11-A8	4	3
301272-7G09-E4	8	16
301272-7G10-E1	62	62
301272-7G11-F6	1	1
301272-7H05-D4	14	20
301272-7H07-C12	42	81
VAT 8C10-8	4	395
VAT 13F5-5	4	5

VAT 12A10-13	11	539
VAT 2C2-1	11	360
REGN472	4	7
REGN474	6	9
REGN475	6	10
REGN476	6	13
REGN477	9	13
Control mAb (RN624)	10	16
Control (TrkA-hFc)	10	15
Control (p75-hFc)	3	5
IgG1 Control	116	116

[0083] The ability of selected test antibodies, REGN472, REGN474, REGN475, REGN476 and REGN477, and control antibody RN624 to block human NGF binding to human TrkA and p75 receptors was also quantitatively measured with a competition sandwich ELISA, in which the presence of the antibody with a fixed concentration of NGF in solution prevented NGF from binding to TrkA-hFc or p75-hFc coated on a microtiter plate. The human NGF used in the assay was a recombinant protein produced in *E. coli* and the human TrkA-hFc and p75-hFc proteins were dimeric fusion proteins consisting of the extracellular domains of the respective receptors fused in-line with the Fc portion of human IgG1. Biotin-labeled NGF protein at a fixed concentration of 50 pM was titrated with various amounts of the antibody from 1.5 pM to 1.5 nM in solution for one hour at room temperature. The amount of unbound free biotin-NGF in the solution mixtures was then quantified by capturing the biotin-NGF on either hTrkA-hFc or hp75-hFc coated microtiter plates, followed by detection of plate bound biotinylated-NGF with Streptavidin-HRP. Specifically, the microtiter plates were prepared by coating the plates with 0.5 µg/ml hTrkA-hFc or 1 µg/ml hp75-hFc solution in PBS buffer overnight at 4°C, followed by blocking the plates with 0.5% BSA prior to use. To measure the unbound biotin-NGF, the pre-incubated antibody and biotin-NGF solutions were transferred to the receptor-coated plates followed by 1-hour incubation at room temperature. The plate-bound biotinylated-NGF was detected with Streptavidin-HRP and developed with a colorimetric TMB substrate, and $OD_{450\,nm}$ recorded. The dependence of the $OD_{450\,nm}$ values on REGN475 concentrations in the pre-binding solutions was analyzed using a sigmoidal dose-response model provided by PRISM™ (Graph Pad, CA). The predicted IC_{50} value, which is defined as the antibody concentration required to block 50% of the binding of 50 pM biotinylated-NGF to the receptor coated plates, was used as an indicator of the potency of the antibody in blocking NGF binding to hTrkA-hFc or hp75-hFc. Table 8 shows IC_{50} values of each antibody tested against hTrkA-hFc and hp75-hFc. Control mAb = RN624.

Table 8

	TrkA-hFc Blocking IC_{50} (pM)	p75-hFc Blocking IC_{50} (pM)
REGN472	12	12
REGN474	8.1	6.3
REGN475	20	22
REGN476	65	61
REGN477	65	62
Control (RN624)	48	72

Example 8. Inhibition of NT-3 Binding to Receptors TrkA-, TrkB-, TrkC- and p75-hFc

[0084] The binding of 20 nM human NT-3 to human TrkA-, TrkB-, TrkC- and p75-hFc surfaces, respectively, in the presence of 500 nM of REGN475, RN624 and AVASTIN® (IgG1 control), was also tested. Human TrkA-hFc (9300 RU), human TrkB-hFc(6000 RU), human TrkC-hFc (9100 RU) and human p75-hFc (7500 RU) were covalently coupled to BIACORE® CM5 chip surfaces by amine-coupling procedure. Twenty nM of human NT-3 was mixed with 500 nM of control (IgG1 control AVASTIN®), REGN475, RN624, hTrkA-hFc, TrkB-hFc, TrkC-hFc or p75-hFc in solution. The binding mixture was first incubated at room temperature to reach equilibrium (about 1 hr) and then was injected over the above TrkA-hFc, TrkB-hFc, TrkC-hFc and p75-hFc surfaces. The level of human NT-3 binding in each sample was measured. The binding RU from each sample mixture was normalized according to the RU value from the negative control sample (*i.e.*, 20 nM human NT-3 with 500 nM AVASTIN®) and presented as % binding to Trk surfaces (Table 9). REGN475 showed almost no interference with NT-3 binding to the receptors, while the remaining samples showed significant blocking of NT-3 binding to the receptors.

Table 9

Antibody	TrkA-hFc	TrkB-hFc	TrkC-hFc	p75-hFc
IgG1 Control	100	100	100	100
RN624	7	8	8	19
REGN475	90	99	101	103
TrkA-hFc	21	5	3	7
TrkB-hFc	6	0	0	0
TrkC-hFc	11	0	0	0
P75-hFc	14	2	2	4

Example 9. Neutralization of NGF Biological Activity *In Vitro*

[0085] The ability of NGF antibodies to block NGF-dependent and TrkA receptor-mediated cell growth activity was carried out using MG87 cells stably transfected with a plasmid encoding human

TrkA receptor. Briefly, the transfected cells were trypsinized and resuspended at approximately 2.5 x 10^5 cells per ml and plated at 5,000 cells per well in a 96-well tissue culture plate. The purified antibody proteins were serially diluted in defined medium plus 0.1% BSA and added to the plated cells at concentrations ranging from 0 to 500 nM. Human NGF was added to the wells to a final concentration of 373 pM. The response was measured after incubating the cells for 3 days at 37°C in a humidified 5% CO_2 incubator. Cell growth activity was measured with a CCK8 kit (Dojindo) and OD_{450nm} recorded. The dependency of the signals on the concentrations of antibody was analyzed and IC_{50} values reported (Table 10, column 2).

[0086] The ability of NGF antibodies to block NGF signaling p75 and TrkA receptor-mediated activity was also measured *in vitro* using a rat adrenal medulla cell line, PC12, which express both receptors endogenously (Urdiales et al. 1998 J. Neuroscience 18(17):6767-6775). Briefly, PC12 cells were stably transfected with a reporter plasmid containing a serum response element (SRE) linked to a luciferase gene. The transfected cells were resuspended at approximately 2.5 x 10^5 cells per ml and plated at 50,000 cells per well in a 96-well tissue culture plate in Opti-MEM media overnight. The purified antibody proteins were serially diluted in medium (DMEM plus 0.1% BSA) and added to the plated cells at concentrations ranging from 0 to 100 nM. Human NGF was added to the wells to a final concentration of 12.5 pM. Luciferase activity was measured after incubating the cells for 6 hours at 37°C in a humidified 7.5% CO_2 incubator using BRIGHT GLOW™ luciferase assay system (Promega). IC_{50} values were determined as described above, and reported in Table 10, column 3. Control mAb = RN624.

Table 10

Antibody	MG87 IC_{50} (nM)	PC12 IC_{50} (nM)
301272-1D07-B10	< 0.186	0.011
301272-1H07-G9	2.000	0.261
301272-1H08-G8	< 0.186	0.006
301272-3D08-C11	0.576	0.005
301272-3F12-D7	< 0.186	—
301272-3G11-C1	< 0.186	0.019
301272-3H10-A10	< 0.186	0.009
301272-3H11-A3	16.000	0.842
301272-6E07-D10	0.293	0.726
301272-6G10-D7	106.000	0.087
301272-7A10-D7	15.000	—
301272-7C05-G1	< 0.186	0.035
301272-7E05-F6	< 0.186	0.018
301272-7F11-A8	0.428	0.071

301272-7G09-E4	3.000	--
301272-7G10-E1	< 0.186	--
301272-7G11-F6	9.000	0.088
301272-7H05-D4	3.000	--
301272-7H07-C12	0.383	0.183
VAT2C2-1	532.000	--
VAT8C10-8	41.000	--
VAT12A10-13	41.000	--
VAT13F5-5	5.000	--
Control (RN624)	< 0.186	0.021

[0087] The ability of selected purified anti-NGF antibodies, REGN472, REGN474, and REGN475, and control mAb RN624 to block NGF signaling through p75 and TrkA receptor-mediated activity in a PC12 cell line was also evaluated with the luciferase assay described above (Table 11).

Table 11

Antibody	IC_{50} (pM)
REGN472	4.5
REGN474	6.6
REGN475	9.6
Control (RN624)	4.9

[0088] The ability of anti-NGF antibody, REGN475, and control antibody to block NT-3 signaling through p75 and TrkA receptor-mediated activity in PC12 cell line was evaluated with the luciferase assay described above, modified by replacing 12.5 pM NGF with 75 nM NT-3. Results showed that the control mAb RN624 blocked NT-3 signaling with an IC_{50} of about 104.8 nM, while REGN475 did not affect NT-3 signaling under the current experimental conditions.

[0089] Further, a bioassay was developed to determine the ability of anti-NGF antibodies, REGN475 and RN624, to neutralize NT-3 mediated cellular function through TrkC *in vitro*. An engineered HEK293 cell line expressing TrkC was transfected with a SRE-luciferase reporter plasmid. NT-3 drives luciferase expression in a 6-hour assay. The ability of REGN475 and RN624 to block NT-3 signaling through TrkC receptor-mediated activity in this engineered cell line was evaluated with the luciferase assay. The engineered HEK293 cell line was seeded onto 96-well plates at 1 $x10^4$ cells/well in serum-free media and incubated overnight at 37°C, 5% CO_2. REGN475 and RN624 at concentrations ranging from 1.6 µM to 28 pM were preincubated with 15 pM NT-3 for 1 hour and the mixture was added to the cells. The cells were then incubated at 37°C, 5% CO_2 for 6 hours. Luciferase activity was determined by adding an equal well volume of BRIGHT GLOW™ (Promega). The result showed that RN624 inhibited NT-3-mediated luciferase

activity with an IC_{50} of ~ 150-200 nM in the presence of a constant concentration of 15 pM NGF, whereas REGN475 did not inhibit NT-3 mediated luciferase activity.

Example 10. Neutralization of NGF Biological Activity *In Vivo*

[0090] Complete Freund's Adjuvant (CFA) test of inflammatory pain. To determine if anti-NGF antibodies could relieve pain in a chronic peripheral inflammatory mouse model, complete Freund's adjuvant (CFA) was injected subcutaneously (s.c.) into the hind paw of C57BL/6 male mice, causing thermal hyperalgesia, which was measured using the Hargreaves' test (Torres et al. (2007) Pain 130:267-278). Control mice received the vehicle (*i.e.*, PBS) only. After acclimating the mice to the Hargreaves' apparatus (model 336, IITC Life Science) for 2-3 hours per day for 3 days, they were tested in the apparatus with an active intensity setting of 17%. A cut-off time of 25 sec was used to avoid tissue damage. For each mouse, 3 readings were obtained during a period of 30 min per day and the median latency was used for analysis. After obtaining a baseline reading in the Hargreaves' apparatus, test anti-NGF antibodies, 301272-7E05-F6 (REGN268) and 301272-7G09-E4 (REGN270), and humanized anti-NGF antibody (RN624) as a positive control, were injected s.c. at 10 mg/kg or 25 mg/kg, 1 hr prior to injecting a 50% solution of CFA (10 mg/20 µl) into the intraplantar hind paw. The Hargreaves' test was repeated daily for up to 4 days after CFA injection and % decrease from the baseline in paw withdrawal latency calculated (Tables 12 and 13, mean % change ± SEM). A significant decrease in thermal hyperalgesia was observed for at least one of the days examined for each of the antibodies tested, compared to control mice that received vehicle only ($p<0.001$-0.05). There was no statistical difference between the tested antibodies and the control antibody. Table 12: n=7 for each group; all groups 10 mg/kg. Table 13: vehicle: n=5; control RN624: n=5, 10 mg/kg; both REGN269: n=9).

Table 12

Time after CFA injection	Vehicle	Control (RN624)	REGN268	REGN270
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-73.8 ± 1.8	-58.3 ± 5.5	-68.3 ± 3.0	-55.8 ± 9.2
Day 2	-67.9 ± 2.1	-30.9 ± 5.2	-44.7 ± 9.5	-36.6 ± 9.9
Day 3	-54.4 ± 2.8	-20.7 ± 6.3	-28.9 ± 11.3	-38.1 ± 5.6

Table 13

Time after CFA injection	Vehicle	Control (RN624)	REGN269 10 mg/kg	REGN269 25 mg/kg
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-82.6 ± 1.6	-61.7 ± 9.7	-79.8 ± 1.8	-80.4 ± 2.2
Day 2	-76.7 ± 3.6	-33.1 ± 17.9	-57.0 ± 8.2	-54.0 ± 5.2

Day 3	-60.8 ± 5.5	-9.6 ± 15.4	-23.9 ± 12.4	-41.1 ± 8.9
Day 4	-40.3 ± 5.0	-0.4 ± 18.5	-25.3 ± 6.6	-16.9 ± 12.6

[0091] Post-operative incision pain model. A rodent model of post-operative pain in which a hind paw plantar incision causes increased sensitivity to touch, guarding behavior, and thermal hyperalgesia, was used to study the efficacy of anti-NGF antibody therapy. For the plantar incision surgery, C57BL/6 mice under isoflurane received an incision through skin, fascia and then isolating the underlying flexor muscle and bisecting vertically. After suture and recovery, the mice were tested for thermal hyperalgesia in the Hargreaves' test and for guarding behavior in the weight bearing test (model 600, IITC Life Science) for 5 days. A single s.c. injection of vehicle (n=7), mAb REGN268 (n=7), or control mAb RN624 (n=7), at 10 mg/kg, was administered 1 hr prior to the incision (Table 14, mean percent change from Hargreaves baseline ± SEM. Table 15 shows results of the weight bearing test (mean percent weight distribution on affected limb ± SEM) (n=7 for each group, control RN624 and REGN268 each 10 mg/kg). In both tests, the pre-treatment with the test antibody or the control antibody significantly reduced the post-operative pain compared to the control mice that received vehicle only ($p<0.001$-0.05).

Table 14

Time after surgery	Vehicle	Control (RN624)	REGN268
Baseline	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Day 1	-72.4 ± 4.4	-62.5 ± 10.6	-59.9 ± 8.9
Day 2	-72.7 ± 3.5	-55.2 ± 9.4	-34.4 ± 21.3
Day 3	-63.8 ± 7.4	-5.3 ± 12.1	-19.8 ± 18.8
Day 4	-52.1 ± 7.8	-6.4 ± 8.7	6.9 ± 4.4
Day 5	-32.7 ± 10.0	-5.3 ± 5.6	6.8 ± 7.8

Table 15

Time after surgery	Vehicle	Control (RN624)	REGN268
Day 0	49.7 ±0.9	49.0 ±0.5	50.3 ±0.7
Day 1	35.3 ±1.5	44.8 ±1.9	39.5 ±3.4
Day 2	34.3 ±1.9	42.7 ±1.8	40.7 ±2.0
Day 3	34.1 ±2.5	48.7 ±1.9	42.0 ±3.3
Day 4	42.2 ±0.8	47.2 ±1.2	44.8 ±1.0
Day 5	48.6 ±1.3	49.7 ±0.7	48.8 ±0.8

[0092] To study whether anti-NGF antibodies could relieve established pain in the post-operative incision pain model, REGN475 (25 mg/kg, n=7), RN624 (25 mg/kg, n=7), and IgG1 control antibody

(25 mg/kg, n=7) were intraperitoneally (i.p.) injected on day 1 post-surgery after performing the behavioral work. Thermal hyperalgesia was studied in the Hargreaves' test and mechanical allodynia was tested in the von Frey test. In this latter test, mice were tested after being acclimated for 2-3 hours for 4 days in an apparatus with a wire mesh floor. The test was performed by applying, in ascending order, a series of von Frey hairs through the wire mesh onto the plantar surface of the hind paw with the incision. A response was considered positive if the paw was raised from the platform in response to application of the filament. Starting from the thinnest hair, each von Frey filament was applied up to five times until a response was observed. The result from the Hargreaves' test (Table 16) showed that the REGN475 antibody treatment led to a significant reversal of the thermal hyperalgesia by 72 hours post-surgery ($p<0.001$-0.01). This return to baseline was not observed in the RN624 treated cohort of mice, which behaved similarly to the IgG control treated group. In the von Frey test (Paw Withdrawal Threshold) (g) (Table 17), both anti-NGF antibodies caused similar relief of mechanical allodynia ($p<0.001$-0.05) (IgG1 control = AVASTIN®, 25 mg/kg, n=7; RN624, 25 mg/kg, n=7; REGN475, 25 mg/kg, n=7).

Table 16

Time after anti-NGF treatment	IgG1 Control	RN624	REGN475
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-66.5 ± 9.0	-74.7 ± 4.3	-72.6 ± 5.4
6 hr	-79.8 ± 3.8	-68.1 ± 5.2	-59.3 ± 9.6
23 hr	-77.6 ± 3.6	-40.5 ± 8.9	-37.0 ± 15.0
47 hr	-61.2 ± 6.6	-37.6 ± 7.2	-30.5 ± 10.8
72 hr	-57.0 ± 7.9	-47.2 ± 8.0	2.1 ± 17.3

Table 17

Time after anti-NGF Treatment	IgG1 Control	RN624	REGN475
Baseline	1.314 ± 0.137	1.314± 0.137	1.286± 0.074
Day 1	0.011 ± 0.002	0.010 ± 0.002	0.010 ± 0.002
5 hr	0.011 ± 0.002	0.083 ± 0.053	0.034 ± 0.009
22 hr	0.029 ± 0.004	0.610 ± 0.123	0.714 ± 0.074
45 hr	0.190 ± 0.135	0.909 ± 0.216	1.086 ± 0.184
70 hr	0.194 ± 0.034	1.143 ± 0.189	1.571 ± 0.437

[0093] On day 4, after the behavioral tests from post incision pain model was completed, the mice's sera were collected and analyzed for circulating levels of neurotrophin-3 (NT-3) using a sandwich ELISA. The limit of detection (~40 pg/ml) was defined as two standard deviations (2σ) above background with a minimum of five NT-3 standards to define the response to concentration curve.

NT-3 levels from mice treated with RN624 (mean ± std dev pg/ml, Table 18) showed a significant increase (172 ± 114 pg/ml, n=7) from those treated with either REGN475 (not detected =ND, n=7) or IgG control (AVASTIN®; ND, n=7).

Table 18

Group	Serum NT-3
RN624	172 ± 114
REGN475	ND
IgG1 control	ND

[0094] For comparison, naïve C57BL/6 mice under isoflurane were given one s.c. injection (50 mg/kg) of REGN475, RN624 or IgG1 control mAb (AVASTIN®) and their sera were analyzed at 1, 7 and 14 days post treatment for NT-3 levels using a sandwich ELISA. The limit of detection (~40 pg/ml) was defined as two standard deviations (2σ) above background with a minimum of five NT-3 standards to define the response to concentration curve. NT-3 levels (Table 19) in RN624 treated mice (131-199 pg/ml, n=6) were elevated compared to REGN475 (ND, n=6) or IgG control (ND, n=6), as observed with the post-operative incision pain model described above.

Table 19

Group	Serum NT-3
Day 1	
RN624	131 ± 41
REGN475	ND
IgG1 Control	ND
Day 7	
RN624	199 ± 15
REGN475	ND
IgG1 Control	ND
Day 14	
RN624	196 ± 35
REGN475	ND
IgG1 Control	ND

[0095] Acute gout joint pain model. A mouse model of joint pain caused by injection of monosodium urate (MSU) crystals into the ankle was used to study the efficacy of the antibodies of the invention to treat gout arthritic joint pain. Endotoxin-free MSU crystals (0.5 mg/20 µl) were injected intra-articularly into the ankle of C57BL/6 mice and the mice were then tested for heel thermal pain in the Hargreaves' test for up to 3 days post-MSU crystals injection. The acclimation parameters and apparatus setting for the Hargreaves' test is as described above. Test mAb 7E05-

F6 (REGN268; n=7), 6E07-D10 (REGN263; n=7), or control humanized mAb (RN624; n=7), or vehicle (n=7) was s.c. injected at 10 mg/kg 1 hr prior to the MSU crystals ankle injection. As shown in Tables 20 and 21, the test antibodies significantly reduced the joint pain, compared to the control mice that received vehicle only ($p<0.001$-0.05).

Table 20

Time after MSU crystals ankle injection	Vehicle	Control (RN624)	REGN268	REGN263
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-62.4 ± 3.1	-33.3 ± 5.2	-28.1 ± 7.8	-36.3 ± 3.8
Day 2	-44.2 ± 3.5	-4.5 ± 11.2	29 ± 19.3	16.8 ± 22.3
Day 3	-24.9 ± 7.9	-3.2 ± 12.0	12.1 ± 15.5	4.5 ± 15.5
Day 4	-11.6 ± 10.5	28.3 ± 18.7	19.9 ± 16.5	-9.0 ± 5.5

Table 21

Time after MSU crystals ankle injection	Vehicle	Control (RN624)	REGN268	REGN263
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-62.6 ± 2.7	-36.0 ± 6.8	-46.7 ± 4.2	-53.9 ± 4.0
Day 2	-54.8 ± 2.7	-11.8 ± 9.8	-28.5 ± 8.4	-35.3 ± 8.5
Day 3	-31.8 ± 3.4	-5.3 ± 8.2	-12.6 ± 9.0	-28.5 ± 8.6

[0096] The ability of an anti-NGF antibody to relieve established pain in the acute gout model was further studied in mice injected with an IL-1 antagonist (IL-1 trap (rinolacept), Economides et al. (2003) Nature 9:47-52) or colchicine. A day after injecting the MSU crystals into the ankles, mice were injected with mIL-1 trap (35 mg/kg; n=7), colchicine (1 mg/kg; n=7), control mAb RN624 (10 mg/kg; n=7), or vehicle (n=7), and tested for thermal hyperalgesia as described above. Additionally, another cohort of mice (n=3) received co-treatment with both mIL-1 trap and the control RN624. Combination therapy of anti-NGF antibody and IL-1 antagonist significantly relieved the established thermal hyperalgesia compared to treatment with vehicle only ($p<0.001$-0.05), or either anti-NGF antibody alone ($p<0.001$) or IL-1 antagonist alone ($p<0.001$) at Day 2 post-treatment (Table 22).

Table 22

Time	Vehicle	mIL-1 Trap	Colchicine	Control (RN624)	mIL-1Trap + RN624
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
Day 1 post MSU injection	-51.9 ± 3.0	-52.9 ± 2.6	-52.8 ± 2.1	-51.9 ± 2.4	-46.6 ± 4.3
7 hr post treatment	-54.8 ± 2.0	-50.6 ± 1.8	-33.1 ± 4.9	-53.2 ± 3.0	-43.3 ± 4.4
Day 1 post treatment	-46.8 ± 2.1	-31.9 ± 6.2	-23.1 ± 7.1	-32.0 ± 10.6	-3.7 ± 11.0

Day 2 post treatment	-37.3 ± 3.6	-9.1 ± 9.4	-23.0 ± 7.6	-27.6 ± 8.3	40.0 ± 29.1
Day 3 post treatment	-26.9 ± 4.4	-12.4 ± 10.4	-14.3 ± 9.8	-9.1 ± 10.9	21.9 ± 19.9

[0100] Neuropathic pain. The mouse Seltzer model of neuropathic pain (Malmberg et al. (1998) Pain 76:215-222) was used with C57BL/6 male mice, in which a partial nerve injury was produced by tying a tight ligature with a 7-0 silk suture around approximately 1/3 to 1/2 the diameter of the sciatic nerve of one single thigh per mouse. Post-surgery, the mice were allowed to recover for at least two days and then they were studied for several weeks post-surgery for thermal hyperalgesia in the Hargreaves' test. Controls were sham-operated mice in which the sciatic nerve was exposed and elevated but not tied. Following surgery, the mice were tested at day 4 and at day 7 post-surgery to confirm that the thermal hyperalgesia had developed. At day 7 post-surgery, the mice were injected s.c. with mAb REGN268 (100 mg/kg), IgG1 control (AVASTIN® 100 mg/kg), and with vehicle. REGN268 significantly relieved established thermal hyperalgesia in this nerve injury model (Table 23; $p<0.05$). This pain relief was not observed in the sham-operated mice. Results expressed as mean percent change from Hargreaves baseline ± SEM (sham-vehicle, n=3; sham-100 mg/kg IgG1 control (AVASTIN®), n=4; sham-100 mg/kg REGN268, n=5; Seltzer-vehicle, n=5; Seltzer-100 mg/kg IgG1 control (AVASTIN®), n=5; Seltzer- 100 mg/kg REGN268, n=8).

Table 23

Days after Surgery	Sham-Vehicle	Sham- IgG1 control	Sham-REGN268	Seltzer-Vehicle	Seltzer-IgG1 control	Seltzer-REGN268
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	8.5 ± 3.9	-7.2 ±3.7	12.0 ± 4.0	-42.5 ± 3.0	-46.9 ± 6.5	-46.4 ± 3.7
7	4.5 ± 1.3	8.7 ± 13.2	8.7 ± 7.7	-45.7 ± 1.5	-55.3 ± 6.4	-46.5 ± 2.7
8	10.3 ±1.7	-9.4 ± 4.1	3.0 ± 5.1	-53.2 ± 3.2	-55.6 ± 4.3	-2.4 ± 6.8
11	5.0 ± 2.3	14.7 ±11.4	1.5 ±5.7	-61.5 ± 4.2	-57.3 ± 5.1	4.2 ± 11.2
13	15.4 ± 2.6	-6.9 ± 3.3	28.7 ± 13.8	-61.4 ± 3.8	-59.7 ± 6.5	1.2 ±5.7
16	4.2 ± 4.0	2.3 ± 3.0	9.0 ± 1.4	-52.2 ± 5.3	-51.2 ± 4.0	2.1 ± 12.1
18	10.2 ± 7.8	0.2 ± 2.8	6.3 ± 4.8	-54.9 ± 4.4	-57.7 ± 4.6	2.2 ± 10.0
20	7.8 ±6.0	3.6 ± 2.4	5.0 ±4.5	-53.8 ±4.5	-53.2 ±4.9	-20.8 ±8.1
24	7.6 ± 5.6	1.5 ± 2.9	11.1 ± 3.2	-56.4 ± 3.0	-54.9 ± 4.1	-26.4 ± 6.8
28	11.1 ± 6.0	0.7± 2.5	10.9 ± 5.0	-53.9 ± 2.1	-51.81 ± 4.1	-5.4 ± 15.0
31	11.7 ± 6.6	1.1 ± 2.3	5.1 ± 1.8	-49.6 ± 4.1	-49.7 ± 2.5	-23.3 ± 11.7

[0101] In the second experiment, in order to see whether anti-NGF treatment could relieve thermal hyperalgesia past day 7 post-surgery, anti-NGF REGN268 (100 mg/kg) was injected s.c. at days 7, 14, or 21 post-surgery. Significant pain relief was obtained at all 3 time points compared to IgG1

control (AVASTIN® 100 mg/kg; $p<0.05$) (Table 24, mean percent change from Hargreaves baseline ± SEM; 100 mg/kg IgG1 control, n=6; 100 mg/kg REGN268, n=7).

Table 24

Days After Surgery	Day 7		Day 14		Day 21	
	IgG1 control	REGN268	IgG1 control	REGN268	IgG1 control	REGN268
Baseline	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
Day 5	-53.5 ±5.9	-51.4 ±5.6	-60.0 ±4.8	-54.2 ±5.2	-55.5 ±5.0	-55.5 ±6.0
Day 7	-55.4 ±4.4	-50.0 ±6.6	-54.4 ±6.4	-47.9 ±3.6	-47.2 ±4.8	-40.7 ±8.5
Day 8	-56.7 ±3.8	17.3 ±13.5	-55.3 ±6.1	-47.2 ±3.2	-47.9 ±4.7	-41.2 ±7.3
Day 10	-64.3 ±2.6	-6.4 ±7.7	-52.8 ±7.2	-62.9 ±5.2	-55.0 ±6.7	-45.9 ±6.9
Day 14	-66.9 ±5.1	-4.9 ±3.4	-62.1 ±5.7	-59.7 ±2.1	-63.8 ±4.6	-61.2 ±3.4
Day 15	-60.6 ±4.0	-1.7 ±10.5	-63.0 ±5.8	-38.5 ±7.3	-54.5 ±5.0	-47.1 ±4.4
Day 17	-58.9 ±3.5	-0.8 ±10.5	-58.6 ±5.7	25.2 ±17.0	-52.4 ±5.3	-48.4 ±4.5
Day 21	-54.1 ±9.6	0.9 ±9.9	-57.1 ±4.4	2.1 ±14.8	-55.8 ±3.6	-48.1 ±5.0
Day 22	-56.3 ±4.9	-1.0 ±10.0	-55.4 ±5.1	-6.4 ±7.1	-50.6 ±5.3	-34.0 ±6.4
Day 24	-55.6 ±5.1	-1.0 ±10.3	-49.5 ±6.6	-2.1 ±11.4	-44.7 ±5.7	-3.2 ±10.8
Day 28	-54.1 ±3.5	-9.0 ±9.6	-53.6 ±5.4	-1.8 ±8.5	-46.0 ±7.6	13.9 ±12.0
Day 32	-41.9 ±8.3	-29.1 ±7.1	-40.9 ±13.1	-10.2 ±8.7	-32.8 ±4.8	8.0 ±12.9
Day 35	-43.9 ±6.8	-32.6 ±7.4	-42.9 ±10.3	-11.8 ±7.9	-39.8 ±4.5	12.2 ±12.4
Day 39	-42.5 ±6.9	-29.0 ±7.9	-39.0 ±11.4	-11.7 ±6.7	-34.6 ±10.0	12.3 ±10.8
Day 42	-35.0 ±6.6	-26.1 ±7.6	-38.1 ±12.5	-8.9 ±8.6	-33.9 ± 9.9	13.5 ±11.5

[0102] In the third experiment, the ability of another anti-NGF antibody REGN475 was tested in the Seltzer model. Following the Seltzer surgery, mice were tested at days 5 and 7 post-surgery to confirm that the thermal hyperalgesia had developed. Then, at day 7 post-surgery the mice were injected by s.c. or i.p. routes with REGN475 (50 mg/kg), control mAb RN624 (50 mg/kg) or IgG1 control (AVASTIN®) (50 mg/kg). Significant pain relief was observed with both anti-NGF antibodies in both cohorts of mice, either injected s.c. (Table 25) or i.p. (Table 26), while control IgG1 showed no effect ($p<0.001$-0.05) (Mean percent change from Hargreaves baseline ± SEM; 50 mg/kg IgG1 control, n=7; 50 mg/kg RN624, n=7; 50 mg/kg REGN475, n=7).

Table 25

Days after Surgery	IgG1 control	RN624	REGN475
Baseline	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

5	-51.2 ±5.2	-53.5 ±6.3	-55.2 ±3.5
7	-47.6 ±3.9	-48.4 ±6.2	-50.9 ±4.3
8	-31.1 ±11.9	4.5 ±9.0	10.3 ±14.1
9	-36.7 ±14.6	-15.2 ±8.5	8.2 ±7.2
12	-47.2 ±5.5	-4.2 ±12.0	-20.1 ±4.3
15	-46.7 ±8.5	2.1 ±10.9	-14.1 ±6.1
19	-28.6 ±7.5	-11.5 ±12.1	3.0 ±10.9
22	-34.9 ±7.9	-5.7 ±9.5	-13.7 ±13.4

Table 26

Days after Surgery	IgG1 control	RN624	REGN475
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	-55.5 ± 3.8	-56.6 ± 2.3	-58.7 ± 2.3
7	-61.6 ± 1.8	-62.3 ± 3.4	-61.7 ± 2.7
8	-59.3 ± 4.0	-3.3 ± 16.0	-8.2 ± 16.1
9	-51.0 ± 4.2	-18.4 ± 12.9	-7.9 ± 9.6
12	-46.5 ± 6.3	-7.0 ± 11.8	-0.1 ± 22.8
15	-43.3 ± 6.6	-16.2 ± 14.8	-10.8 ± 18.0

[0103] To determine the ability of antibodies to neutralize human NGF activities *in vivo*, transgenic mice were made of which the endogenous mouse NGF locus was replaced with the human NGF gene. These mice were used in a Seltzer neuropathic pain model to test REGN268 and control mAbs. Following the Seltzer surgery, these mice were tested at day 4 and at day 8 post-surgery to confirm that the thermal hyperalgesia had developed. Then, at day 8 post-surgery the mice were injected s.c. with 50 mg/kg mAb REGN268 (n=7), 50 mg/kg control RN624 (n=8), or 50 mg/kg IgG1 control (AVASTIN®) (n=6). The results (Table 27) showed that REGN268 was as efficacious as a humanized anti-NGF antibody (RN624) in relieving the neuropathic pain in the humanized NGF mice, whereas IgG1 control had no effect ($p<0.05$).

Table 27

Days after Surgery	IgG1 Control	RN624	REGN268
Baseline	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8	-55.4 ± 5.7	-38.1 ± 6.4	-40.8 ± 6.9
10	-54.3 ± 8.0	-23.0 ± 7.7	-16.8 ± 5.4
12	-44.8 ± 7.9	-18.4 ± 8.4	-15.1 ± 10.1

14	-41.3 ± 7.0	5.0 ± 23.6	-6.7 ± 12.9
16	-42.5 ± 8.8	-12.7 ± 9.5	5.2 ± 16.3
20	-44.2 ± 8.9	-15.7 ± 13.0	-8.0 ± 13.7

Example 11. Effect of Anti-NGF on Animal Motor Function

[0104] In order to study whether anti-NGF treatment could alter motor function, motor coordination in the rotarod test in naive C57BL/6 male mice was assessed. Animals were first trained to stay on a rotarod (Columbus Instruments, 3.5 cm diameter, 9 cm wide) rotating at progressively higher speeds (maximum speed 10 rpm). Mice remained at 10 rpm in training until they could walk for 60 sec consecutively, or until they had spent a total of 2 min walking on the rotarod at 10 rpm each day for three consecutive days. After training, each mouse was placed on the rotarod at 10 rpm three times consecutively (with a brief break between trials), and the latency to fall off was recorded. Animals were removed after 1 min, and assigned a score of 60 sec if they did not fall off. The median score of 3 trials for each mouse was used in analysis. After obtaining a baseline reading in the rotarod, mAbs REGN475, RN624, or IgG negative control was injected s.c. at 50 mg/kg or 100 mg/kg. The mice were then tested for up to 20 days post-antibody injection. Results (Table 28, expressed as latency to fall in sec) (mean ± sem) showed that the mice treated with RN624, but not REGN475, had significantly impaired motor coordination ($p<0.001$-0.05). Interestingly, it has been reported that NT-3 and TrkC knock-out mice displayed abnormal movements and postures and lost proprioception (Ernfors et al. (1994) Cell 77:503-512; and Klein et al. (1994) Nature 368:249-251). Besides the rotarod, Hargreaves' and von Frey tests on naïve mice injected with anti-NGF antibodies were also conducted. No statistically significant differences were observed for any group of mice in the Hargreaves' and von Frey tests during the 20 days post-antibody administration (n=6 for each group).

Table 28

Time after anti-NGF treatment	100mg/kg IgG1 control	50mg/kg RN624	100mg/kg RN624	50mg/kg REGN475	100mg/kg REGN475
Baseline	57.7 ±1.6	54.2 ±4.6	60.0 ±0.0	55.0 ±3.6	60.0 ±0.0
Day 1	59.2 ±0.7	43.2 ±3.7	32.8 ±2.2	58.0 ±1.3	58.4 ±1.5
Day 4	52.8 ±4.8	36.3 ±4.3	32.5 ±3.2	52.5 ±4.3	53.2 ±3.3
Day 7	57.7 ±1.8	47.2 ±3.7	37.5 ±5.2	58.0 ±1.3	60.0 ±0.0
Day 11	58.7 ±1.0	50.0 ±4.7	44.7±6.2	55.2 ±2.1	60.0 ±0.0
Day 15	57.8 ±1.6	56.7 ±2.6	36.0 ±1.7	55.2 ±2.2	57.7 ±1.6
Day 20	57.8±1.8	57.8 ±1.3	45.7 ±5.0	51.8 ±3.3	53.7 ±3.1

Example 12. Treatment of Patient Suffering from Post-Herpetic Neuralgia

[0105] A patient who has developed chronic pain at the site of a shingles rash is diagnosed with post-herpetic neuralgia. The patient is treated by administration of therapeutically-effective amount of a pharmaceutically acceptable composition comprising an anti-NGF mAb of the invention. Administration may be by subcutaneous or intravenous injection, at the anti-NGF antibody concentrations of, preferably, between 0.1 to 10 mg/kg body weight. Frequency of treatment may be every 1 -12 weeks, or as needed. Within several days after administration of the anti-NGF antibody composition, the patient's pain is substantially alleviated. Repeated administration of the anti-NGF mAb composition maintains this pain relief.

Example 13. Treatment of Patient Suffering from Osteoarthritis Pain

[0106] A patient suffering from moderate to severe pain caused by osteoarthritis in any joint is treated by administering the therapeutically-effective amount of a pharmaceutically acceptable composition comprising an anti-NGF mAb of the invention. The composition can be administered intravenously at the concentrations of the anti-NGF antibody between 10 μg/kg body weight to 10 mg/kg body weight. Frequency of treatment may be every 1-12 weeks, or as needed. Within several days of the administration of the anti-NGF antibody composition, the patient's pain is substantially alleviated and regain mobility of the affected joint. The treatment can be repeated as long as necessary.

We claim:

1. A human antibody or antigen-binding fragment of an antibody that specifically binds human nerve growth factor (NGF) with K_D of 5 pM or less, as measured by surface plasmon resonance, wherein the antibody or fragment thereof binds human NGF with a K_D of about 2 to about 10-fold higher than the antibody or fragment binds rat and mouse NGF.

2. A human antibody or antigen-binding fragment according to claim 1,wherein the antibody or antibody fragment comprises a heavy chain complementary determining region 3 (HCDR3) and a light chain CDR3 (LCDR3), wherein the HCDR3 and LCDR3 comprise amino acid sequences that have at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:90 and 98, respectively.

3. The human antibody or antigen-binding fragment according to claim 2, further comprising a HCDR1, HCDR2, LCDR1 and LCDR2, wherein the HCDR1 is SEQ ID NO:86, HCDR2 is SEQ ID NO:88, LCDR1 is SEQ ID NO:94 and LCDR2 is SEQ ID NO:96.

4. A human antibody or antigen-binding fragment of an antibody that specifically binds human nerve growth factor (NGF) with K_D of 5 pM or less, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR and LCVR are amino acid sequences comprising at least 90% sequence identity to the amino acid sequences set forth in SEQ ID NO:108 and 110, or SEQ ID NO:100 and 102.

5. A human antibody or antigen-binding fragment of an antibody that specifically binds human NGF with K_D of 5 pM, comprising HCDR3 and LCDR3,

wherein HCDR3 comprises an amino acid sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9 – X^{10} – X^{11} - X^{12} – X^{13} – X^{14} – X^{15} – X^{16} – X^{17} – X^{18}$ (SEQ ID NO:537) wherein X^1 is Ala or Ser, X^2 is Thr or Lys, X^3 is Glu or Ile, X^4 is Phe or Gly, X^5 is Val or Gly, X^6 is Val or Trp, X^7 is Val or Phe, X^8 is Thr or Gly, X^9 is Asn or Lys, X^{10} is Phe or Leu, X^{11} is Asp or Phe, X^{12} is Asn or Ser, X^{13} is Ser or absent, X^{14} is Tyr or absent, X^{15} is Gly or absent, X^{16} is Met or absent, X^{17} is Asp or absent, and X^{18} is Val or absent; and

LCDR3 comprises an amino acid sequence of the formula $X^1 – X^2 – X^3 – X^4 – X^5 – X^6 – X^7 – X^8 – X^9$ (SEQ ID NO:540) wherein X^1 is Gln, X^2 is Gln, X^3 is Tyr, X^4 is Asn, X^5 is Arg or Asn, X^6 is Tyr or Trp, X^7 is Pro, X^8 is Tyr or Trp, and X^9 is Thr.

6. A human antibody or antibody fragment according to claim 5, further comprising HCDR1, HCDR2, LCDR1 and LCDR2, wherein

HCDR1 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:535), wherein X^1 is Gly, X^2 is Phe, X^3 is Thr or Asn, X^4 is Phe or Leu, X^5 is Thr or Asp, X^6 is Asp or Glu, X^7 is Tyr or Leu, and X^8 is Ser or Ala;

HCDR2 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:536), wherein X^1 is Ile or Phe, X^2 is Asp or Ser, X^3 is Pro or Trp, X^4 is Glu or Asn, X^5 is Asp or Ser, X^6 is Gly, X^7 is Thr, Glu or Ser, X^8 is Thr or Ile;

LCDR1 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6$ (SEQ ID NO:538) wherein X^1 is Gln or Arg, X^2 is Ala, Ser or Thr, X^3 is Val or Ile, X^4 is Arg or Thr, X^5 is Asn, Phe or Tyr, and X^6 is Asp or Asn; and

LCDR2 comprises an amino acid sequence of the formula $X^1 - X^2 - X^3$ (SEQ ID NO:539) wherein X^1 is Gly or Ala, X^2 is Ala, and X^3 is Ser or Phe.

7. A nucleic acid molecule encoding the human antibody or antigen-binding fragment of an according to any one of claims 1 to 6.

8. An expression vector comprising the nucleic acid molecule according to claim 7.

9. A method of producing an anti-human NGF antibody or antigen-binding fragment of an antibody comprising the steps of introducing the expression vector of claim 8 into an isolated host cell, growing the cell under conditions permitting production of the antibody or antibody fragment, and recovering the antibody or antibody fragment so produced.

10. A method according to claim 8, wherein the host cell is an *E. coli* cell, a CHO cell, or a COS cell.

11. A pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 6 and a pharmaceutically acceptable carrier.

12. A pharmaceutical composition according to claim 11 further comprising a further therapeutic agent selected from an interleukin-1 (IL-1) inhibitor, an antiepileptic drug, a cytokine antagonist, and another neurotrophin.

13. An antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 6 for use to attenuate or inhibit a NGF-mediated disease or condition in a human.

14. An antibody or antigen-binding fragment of an antibody according to claim 11 for use in combination with a further therapeutic agent selected from an interleukin-1 (IL-1) inhibitor, an

antiepileptic drug, a cytokine antagonist, and another neurotrophin.

15. An antibody or antigen-binding fragment of an antibody according to claim 13 or 14, wherein the NGF-mediated condition or disease is inhibited without significant impairment of motor coordination.

16. An antibody or antigen-binding fragment of an antibody according to any one of claims 12 to 15, wherein the NGF-mediated condition or disease is selected from inflammatory pain, post-operative incision pain, neuropathic pain, fracture pain, gout joint pain, post-herpetic neuralgia, pain resulting from burns, cancer pain, osteoarthritis or rheumatoid arthritis pain, sciatica, pain associated with sickle cell crises, or post-herpetic neuralgia.

17. Use of an antibody or antigen-binding fragment of an antibody according to any one of claim 1 to 6 in the manufacture of a medicament for use to attenuate or inhibit an NGF-mediated disease or condition in a human.

18. Use according to claim 17, wherein the NGF-mediated condition or disease is inhibited without significant impairment of motor coordination.

19. Use according to claim 17, wherein the NGF-mediated condition or disease is selected from inflammatory pain, post-operative incision pain, neuropathic pain, fracture pain, gout joint pain, post-herpetic neuralgia, pain resulting from burns, cancer pain, osteoarthritis or rheumatoid arthritis pain, sciatica, pain associated with sickle cell crises, or post-herpetic neuralgia.

20. Use according to any one of claims 17 to 19 in the manufacture of a medicament for use in combination with a further therapeutic agent selected from an interleukin-1 (IL-1) inhibitor, an antiepileptic drug, a cytokine antagonist, and another neurotrophin.

21. A method of treating an NGF-related condition or disease which is inhibited, ameliorated, or reduced by inhibition of NGF, comprising administering a first and a second therapeutic agent to a human subject in need thereof, wherein the first therapeutic agent is an antibody or antigen-binding fragment of an antibody which specifically binds human nerve growth factor (NGF) with K_D of 5 pM as measured by surface plasmon resonance, wherein the antibody or fragment thereof binds human NGF with a K_D of about 2 to about 10-fold higher than the antibody or fragment binds rat and mouse NGF.

22. A method according to claim 21, wherein said antibody or antigen-binding fragment is an antibody or antigen-binding fragment of any one of claims 1 to 5.

23. A method according to claim 21 or 22, wherein the second therapeutic agent is an interleukin-1 (IL-1) inhibitor, an antiepileptic drug, a cytokine antagonist, or another neurotrophin.

24. A method according to any one of claims 21 to 23, wherein the NGF-mediated condition or disease is inhibited without significant impairment of motor coordination.

25. A method according to any one of claims 21 to 24, wherein the NGF-mediated condition or disease is selected from inflammatory pain, post-operative incision pain, neuropathic pain, fracture pain, gout joint pain, post-herpetic neuralgia, pain resulting from burns, cancer pain, osteoarthritis or rheumatoid arthritis pain, sciatica, pain associated with sickle cell crises, or post-herpetic neuralgia.