Human anti-NGF Neutralizing Antibodies as Selective NGF Pathway Inhibitors

This application claims priority to U.S. patent application Serial No. (U.S.S.N.) 12/576,522, filed October 9, 2009, which is a continuation-in-part application of U.S. patent application Serial No. (U.S.S.N.) 12/277,919, filed November 25, 2008, which is a continuation application of U.S. patent application Serial No. (U.S.S.N.) 10/891,658, filed July 15, 2004, and claims the benefit of priority to U.S. provisional application Serial No. 60/487,431, filed July 15, 2003. This application is also related to U.S. patent application Serial No. 11/767,326, filed June 22, 2007, which is a divisional application of U.S.S.N. 10/891,658. The disclosures of all these applications are incorporated by reference herein.

The sequence listing is filed with the application in electronic format only and is incorporated by reference herein. The sequence listing text file "02-1240-F-CIP.SeqList.txt" was created on November 25, 2008, and is 79,116 bytes in size.

FIELD OF THE INVENTION

The invention relates to human monoclonal antibodies that bind nerve growth factor (NGF). Compositions and methods for treating pain and pain-related disorders are also described.

BACKGROUND OF THE INVENTION

Every day, more than two million people in the United States are incapacitated by chronic pain (Jessell and Kelly, 1991, "Pain and Analgesia" in PRINCIPLES OF NEURAL SCIENCE, 3rd Ed., (Kandel, Schwartz, and Jessell, ed.), Elsevier, New York). Unfortunately, current treatments for pain are only partially effective, and many of these treatments themselves cause debilitating or dangerous side effects. For example, although non-steroidal anti-inflammatory drugs ("NSAIDs") such as aspirin, ibuprofen, and indomethacin are moderately effective against inflammatory pain, they are also renal toxins, and high doses tend to cause gastrointestinal irritation, ulceration, bleeding, and mental confusion. Patients treated with opioids also frequently experience confusion, and long-term opioid use is associated with

to their use for treating and managing pain, the antibodies of the invention are useful for treating neuropathic and inflammatory pain-related responses.

The invention further provides fusion proteins comprising the sequence of an antibody Fc region and one or more sequences identified as SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NOs: 79-130. Such molecules can be prepared using methods as described, for example, in International Patent Application, Publication No. WO 00/24782, which is incorporated by reference. Such molecules can be expressed, for example, in mammalian cells (*e.g.* Chinese Hamster Ovary cells) or bacterial cells (*e.g. E. coli* cells).

In certain aspects, the invention provides antibodies, preferably monoclonal antibodies, most preferably human antibodies and human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof and the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 4.

In certain aspects, the invention provides antibodies, preferably human antibodies, and more preferably monoclonal antibodies, most preferably human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE heavy chain constant regions or any allelic variation thereof (as discussed in Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), included herein by reference, and the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, an antibody of the invention comprises an amino acid sequence of the IgG2 heavy chain constant region as set forth in SEQ ID NO: 4 or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 11 shows anti-NGF light chain alignment and percent identity for the 14D10 (SEQ ID NO: 82), 6H9 (SEQ ID NO: 84), 7H2 (SEQ ID NO: 86), 4G6a (SEQ ID NO: 88), 4G6b (SEQ ID NO: 89), 4G6c (SEQ ID NO: 90), 4G6d (SEQ ID NO: 91), 4G6e (SEQ ID NO: 131), 14D11 (SEQ ID NO: 80), and 4D4 (SEQ ID NO: 12) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 12 shows anti-NGF heavy chain alignment and percent identity for the 4D4 (SEQ ID NO: 10), 4G6 (SEQ ID NO: 87), 14D10 (SEQ ID NO: 81), 14D11 (SEQ ID NO: 79), 7H2 (SEQ ID NO: 85), and 6H9 (SEQ ID NO: 83) antibodies.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein for any purpose.

Definitions

Conventional techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.*, 2001, MOLECULAR CLONING: A LABORATORY MANUAL, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference for any purpose. Unless specific definitions are provided, the nomenclature utilized in connection with, and

The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. *See, e.g.*, LaPlanche *et al.*, 1986, *Nucl. Acids Res.*, 14:9081; Stec *et al.*, 1984, *J. Am. Chem. Soc.*, 106:6077; Stein *et al.*, 1988, *Nucl. Acids Res.*, 16:3209; Zon *et al.*, 1991, *Anti-Cancer Drug Design*, 6:539; Zon *et al.*, 1991, OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, 1990, *Chemical Reviews*, 90:543, the disclosures of which are hereby incorporated by reference for any purpose. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e. g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell. and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The GAP program may be useful with the above parameters. In certain embodiments, the aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

The term "homology" refers to the degree of similarity between protein or nucleic acid sequences. Homology information is useful for the understanding the genetic relatedness of certain protein or nucleic acid species. Homology can be determined by aligning and comparing sequences. Typically, to determine amino acid homology, a protein sequence is compared to a database of known protein sequences. Homologous sequences share common functional identities somewhere along their sequences. A high degree of similarity or identity is usually indicative of homology, although a low degree of similarity or identity does not necessarily indicate lack of homology.

Several approaches can be used to compare amino acids from one sequence to amino acids of another sequence to determine homology. Generally, the approaches fall into two categories: (1) comparison of physical characteristics such as polarity, charge, and Van der Waals volume, to generate a similarity matrix; and (2) comparison of likely substitution of an amino acid in a sequence by any other amino acid, which is based on observation of many protein sequences from known homologous proteins and to generate a Point Accepted Mutation Matrix (PAM).

The percentage of identity may also be calculated by using the program needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 9.0.0 software package, using the default parameters (for example, GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See IMMUNOLOGY--A SYNTHESIS, 2nd Edition, (E. S. Golub and D. R. Gren, Eds.), Sinauer Associates: Sunderland, MA, 1991, incorporated herein by reference for any purpose. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids; unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-

51: 1-84; Ravetch and Bolland, 2001, *Annu. Rev. Immunol.* 19: 275-90; Shields *et al.*, 2001, *Journal of Biol. Chem* 276: 6591-6604; Telleman and Junghans, 2000, *Immunology* 100: 245-251; Medesan *et al.*, 1998, *Eur. J. Immunol.* 28: 2092-2100; all of which are incorporated herein by reference). Such mutations can include substitutions, additions, deletions, or any combination thereof, and are typically produced by site-directed mutagenesis using one or more mutagenic oligonucleotide(s) according to methods described herein, as well as according to methods known in the art (see, *for example*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 3rd Ed., 2001, Cold Spring Harbor, N.Y. and Berger and Kimmel, METHODS IN ENZYMOLOGY, Volume 152, Guide to Molecular Cloning Techniques, 1987, Academic Press, Inc., San Diego, CA., which are incorporated herein by reference).

According to certain embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (5) confer or modify other physicochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In preferred embodiments, a conservative amino acid substitution typically does not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in PROTEINS, STRUCTURES AND MOLECULAR PRINCIPLES, (Creighton, Ed.), 1984, W. H. Freeman and Company, New York; INTRODUCTION TO PROTEIN STRUCTURE (C. Branden and J. Tooze, eds.), 1991, Garland Publishing, New York, N.Y.; and Thornton *et al.*, 1991, *Nature* 354:105, each of which are incorporated herein by reference.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". *See*

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

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. In certain embodiments, binding fragments are produced by recombinant DNA techniques. In additional embodiments, binding fragments are produced by enzymatic or chemical cleavage of intact antibodies. Binding fragments include, but are not limited to, F(ab), F(ab'), $F(ab')_2$, Fv, and single-chain antibodies.

The term "heavy chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. The term "light chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. A full-length heavy chain includes a variable region domain, V_H, and three constant region domains, C_H1, C_H2, and C_H3. The V_H domain is at the amino-terminus of the polypeptide, and the C_H3 domain is at the carboxyl-terminus. The term "heavy chain", as used herein, encompasses a full-length heavy chain and fragments thereof. A full-length light chain includes a variable region domain, V_L, and a constant region domain, C_L. Like the heavy chain,

antibodies or other previously described non-fully human antibodies from non-human species.

One skilled in the art can engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci so that such mice produce human antibodies in the absence of mouse antibodies. Large human Ig fragments may preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse cellular machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains yields high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity may be produced and selected.

Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-2555, (1993); Jakobovits et al., Nature, 362:255-258, (1993; Bruggemann et al., Year in Immun., 7:33 (1993); Nature 148:1547-1553 (1994), Nature Biotechnology 14:826 (1996); Gross, J.A., et al., Nature, 404:995-999 (2000); and U.S. patents nos. 5,877,397, 5,874,299, 5,814,318, 5,789,650, 5,770,429, 5,661,016, 5,633,425, 5,625,126, 5,569,825, and 5,545,806 (each of which is incorporated herein by reference in its entirety for all purposes)). Human antibodies can also be produced in phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1992); Marks et al., J. Mol. Biol., 222:581 (1991)). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therap, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(l):86-95 (1991)).

Recombinant human antibodies may also be 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 those related to human

germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

In certain embodiments, the skilled artisan can use constant regions from species other than human along with the human variable region(s) in such mice to produce chimeric antibodies.

Naturally Occurring Antibody Structure

Naturally occurring antibody structural units typically comprise a tetramer. Each such tetramer typically is composed of two identical pairs of polypeptide chains, each pair having one full-length "light" chain (typically having a molecular weight of about 25 kDa) and one full-length "heavy" chain (typically having a molecular weight of about 50-70 kDa). The amino-terminal portion of each light and heavy chain typically includes a variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant region responsible for effector function. Human light chains are typically classified as kappa and lambda light chains. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA is similarly subdivided into subclasses including, but not limited to, IgA1 and IgA2. Within full-length light and heavy chains, typically, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See, e.g.*, FUNDAMENTAL IMMUNOLOGY, Ch. 7, 2nd ed., (Paul, W., ed.), 1989, Raven Press, N.Y. (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair typically form the antigen-binding site.

The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain

amino acids are specific amino acids that are in positions in which they are not usually found in FRs. The grafted variable regions from anti-NGF antibodies of the invention can be used with a constant region that is different from the constant region of anti-NGF antibody. Alternatively, the grafted variable regions are part of a single chain Fv antibody. CDR grafting is described, *e.g.*, in U.S. Patent Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101, which are hereby incorporated by reference for any purpose.

Antibodies of the invention are preferably prepared using transgenic mice that have a substantial portion of the human antibody producing locus inserted in antibody-producing cells of the mice, and that are further engineered to be deficient in producing endogenous, murine, antibodies. Such mice are capable of producing human immunoglobulin molecules and antibodies and do not produce or produce substantially reduced amounts of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving this result are disclosed in the patents, applications, and references disclosed in the specification herein. In preferred embodiments, the skilled worker may employ methods as disclosed in International Patent Application Publication No. WO 98/24893, which is hereby incorporated by reference for any purpose. *See also* Mendez *et al.*, 1997, *Nature Genetics* 15:146-156, which is hereby incorporated by reference for any purpose.

The monoclonal antibodies (mAbs) of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology, *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975, *Nature* 256:495). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies can be employed, *e.g.*, viral or oncogenic transformation of B-lymphocytes.

The preferred animal system for preparing hybridomas is the mouse. Hybridoma production in the mouse is very well established, and immunization protocols and techniques for isolation of immunized splenocytes for fusion are well known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

In a preferred embodiment, human monoclonal antibodies directed against NGF can be generated using transgenic mice carrying parts of the human immune

system rather than the mouse system. These transgenic mice, referred to herein as "HuMab" mice, contain a human immunoglobulin gene minilocus that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg *et al.*, 1994, *Nature* 368:856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ and in response to immunization, the introduced human heavy chain and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgG κ monoclonal antibodies (Lonberg *et al., supra.*; Lonberg and Huszar, 1995, *Intern. Rev. Immunol.* 13:65-93; Harding and Lonberg, 1995, *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation of HuMab mice is described in detail in Taylor *et al.*, 1992, *Nucleic Acids Res.* 20:6287-6295; Chen *et al.*, 1993, *International Immunology* 5:647-656; Tuaillon *et al.*, 1994, *J. Immunol.* 152:2912-2920; Lonberg *et al.*, 1994, *Nature* 368:856-859; Lonberg, 1994, *Handbook of Exp. Pharmacology* 113:49-101; Taylor *et al.*, 1994, *International Immunology* 6:579-591; Lonberg & Huszar, 1995, *Intern. Rev. Immunol.* 13:65-93; Harding & Lonberg, 1995, *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild *et al.*, 1996, *Nature Biotechnology* 14:845-851, the contents of all of which are hereby incorporated by reference in their entirety. *See further* U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, as well as U.S. Patent No. 5,545,807 to Surani *et al.*; International Patent Application Publication Nos. WO 93/1227, published June 24, 1993; WO 92/22646, published December 23, 1992; and WO 92/03918, published March 19, 1992, the disclosures of all of which are hereby incorporated by reference in their entirety. Alternatively, the HCo7, HCo12, and KM transgenic mice strains described in the Examples below can be used to generate human anti-NGF antibodies.

The present invention provides human monoclonal antibodies that are specific for and neutralize bioactive human NGF polypeptides. Also provided are antibody heavy and light chain amino acid sequences which are highly specific for and neutralize NGF polypeptides when they are bound to them. This high specificity enables the anti-human NGF human antibodies, and human monoclonal antibodies with like specificity, to be effective immunotherapy for NGF associated diseases.

In one aspect, the invention provides isolated human antibodies that bind the same or essentially the same epitope as the 4D4 antibody provided herein.

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of anti-NGF antibody, or to increase or decrease the affinity of the anti-NGF antibodies described herein.

As it is well known, minor changes in an amino acid sequence such as deletion, addition or substitution of one, a few or even several amino acids may lead to an allelic form of the original protein which has substantially identical properties. Therefore, in addition to the antibodies specifically described herein, other "substantially homologous" antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis. Therefore, the present invention contemplates "variant" or "mutant" anti-NGF human antibodies having substantially similar characteristics to the anti-NGF human antibodies disclosed herein (See, for example, WO 00/56772, all of which is hereby incorporated herein by reference). Thus, by the term "variant" or "mutant" in reference to an anti-NGF human antibody is meant any binding molecule (molecule X) (i) in which the hypervariable regions CDR1, CDR2, and CDR3 of the heavy chain or the hypervariable regions CDR1, CDR2, and CDR3 of the light chain taken as a whole are at least 80% homologous, preferably at least 90% homologous, more preferably at least 95% homologous to the hypervariable regions as shown in SEQ ID NOS: 14, 18, and 22 or SEQ ID NOS: 16, 20, and 24, respectively, and (ii) wherein the variant or mutant is capable of inhibiting the activity of human NGF to the same extent as a reference anti-NGF human antibody having framework regions identical to those of molecule X.

Ordinarily, an anti-NGF human antibody variant will have light and/or heavy chain CDRs, when taken as a whole, that are at least about 80% amino acid sequence identity, preferably at least about 85% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet

The sequences of the 4D4 antibody heavy chain and light chain variable regions are shown in SEQ ID NOS: 10 and 12, respectively. However, many of the potential CDR-contact residues are amenable to substitution by other amino acids and still allow the antibody to retain substantial affinity for the antigen. Likewise, many of the framework residues not in contact with the CDRs in the heavy and light chains can accommodate substitutions of amino acids from the corresponding positions from other human antibodies, by human consensus amino acids, or from other mouse antibodies, without significant loss of the affinity or non-immunogenicity of the human antibody. Selection of various alternative amino acids may be used to produce versions of the disclosed anti-NGF antibodies and fragments thereof that have varying combinations of affinity, specificity, non-immunogenicity, ease of manufacture, and other desirable properties.

In alternative embodiments, antibodies of the invention can be expressed in cell lines other than hybridoma cell lines. In these embodiments, sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. According to these embodiments, transformation can be achieved using any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (all of which are hereby incorporated herein by reference for any purpose). Generally, the transformation procedure used may depend upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

A nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of an NGF antibody of the invention is inserted into an appropriate expression vector using standard ligation techniques. In a preferred embodiment, the anti-NGF antibody heavy chain or light chain constant region is appended to the C-terminus of the appropriate variable region and is ligated into an

sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide controlled or sustained release of the product which can be delivered *via* depot injection. In certain embodiments, hyaluronic acid may also be used, having the effect of promoting sustained duration in the circulation. In certain embodiments, implantable drug delivery devices may be used to introduce the desired antibody molecule.

Pharmaceutical compositions of the invention can be formulated for inhalation. In these embodiments, anti-NGF antibodies are advantageously formulated as a dry, inhalable powder. In preferred embodiments, anti-NGF antibody inhalation solutions may also be formulated with a propellant for aerosol delivery. In certain embodiments, solutions may be nebulized. Pulmonary administration and formulation methods therefore are further described in International Patent Application No. PCT/US94/001875, which is incorporated by reference and describes pulmonary delivery of chemically modified proteins.

It is also contemplated that formulations can be administered orally. Anti-NGF antibodies that are administered in this fashion can be formulated with or without carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In certain embodiments, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the anti-NGF antibody. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

A pharmaceutical composition of the invention is preferably provided to comprise an effective quantity of one or a plurality of anti-NGF antibodies in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving anti-NGF antibodies in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. *See, for example*, International Patent Application No. PCT/US93/00829, which is incorporated by reference and describes controlled release of porous polymeric microparticles for delivery of pharmaceutical compositions. Sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (as disclosed in U.S. Patent No. 3,773,919 and European Patent Application Publication No. EP 058481, each of which is incorporated by reference), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-556), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-105), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent Application Publication No. EP 133,988). Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art. See *e.g.*, Eppstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-3692; European Patent Application Publication Nos. EP 036,676; EP 088,046 and EP 143,949, incorporated by reference.

Pharmaceutical compositions used for *in vivo* administration are typically provided as sterile preparations. Sterilization can be accomplished by filtration through sterile filtration membranes. When the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. Compositions for parenteral administration can be stored in lyophilized form or in a solution. Parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) that is reconstituted prior to administration.

harvested by centrifugation at four hours post induction. Final OD was 75. Expression yield was determined to be approximately 0.15 g/L.

Refolding and purification of rHu-NGF(1-120)

Cell paste was lysed in a Microfluidizer, centrifuged at 10,000 X g for 30 minutes, the pellet was washed with 1% deoxycholic acid, centrifuged as above, and the resulting pellet was then washed with cold water and re-centrifuged. The resulting pellet (WIBs – washed inclusion bodies) was resuspended in denaturant, 8M guanidine HCl, 50mM Tris pH 8.5, containing 10mM DTT, and solubilized at room temperature for 1 hour, centrifuged at 10,000 x g for 30 minutes, and the supernatent was carefully decanted and then diluted 25-fold into an aqueous buffer containing a redox couple at 4°C, for 5 days. The resulting refold was then titrated to pH 3.0, filtered through a 0.45uM filter. The refold was purified using a Sp-Sepharose fast flow column using a standard NaCl gradient. The pool from the cation exchange column was subsequently concentrated and aliquots were frozen –80°C. The purity of the protein was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Coomassie blue stain. The purified protein was greater than 90% main band by this method.

Example 2

Production of Human Monoclonal Antibodies Against Nerve Growth Factor (NGF)

Transgenic HuMab and KM Mice

Fully human monoclonal antibodies to NGF were prepared using HCo7, HCo12, HCo7+HCo12, and KM strains of transgenic mice, each of which expresses human antibody genes. In each of these mouse strains, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993, *EMBO J.* 12:811-820), and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of International Patent Application Publication No. WO 01/09187 (incorporated by reference). Each of these mouse strains carries a human kappa light chain transgene, KCo5, as described in

Fishwild *et al.* (1996, *Nature Biotechnology* 14:845-851). The HCo7 strain carries the HCo7 human heavy chain transgene as described in U.S. Patent Nos. 5,545,806, 5,625,825, and 5,545,807 (incorporated by reference). The HCo12 strain carries the HCo12 human heavy chain transgene as described in Example 2 of International Patent Application Publication No. WO 01/09187 (incorporated by reference). The HCo7+HCo12 strain carries both the HCo7 and the HCo12 heavy chain transgenes and is hemizygous for each transgene. The KM mice comprises the SC20 heavy chain transgene as described in Tomizuka et al. (1997, *Nature Genet.* 16, 133-143 and 2000, *Proc. Natl. Acad. Sci*, 97, 722-727). This transgene is not integrated into a mouse chromosome, but is instead propagated as an independent chromosome fragment. The fragment includes approximately 15 MB of human chromosome 14. It contains the entire human heavy chain locus including all VH, D and JH gene segments and all heavy chain constant region isotypes. All of these strains are referred to herein as HuMab mice.

HuMab Immunizations:

To generate fully human monoclonal antibodies to NGF, HuMab mice were immunized with purified recombinant NGF derived from *E. coli* cells as antigen (Example 1). General immunization schemes for HuMab mice are described in Lonberg *et al.* (1994, *Nature* 368:856-859; Fishwild *et al.*, *supra.*, and International Patent Application Publication No. WO 98/24884, the teachings of each of which are incorporated by reference). Mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (25-100 μg) of NGF antigen was used to immunize the HuMab mice intraperitoneally (IP) or subcutaneously (SC).

Immunizations of HuMab transgenic mice were achieved using antigen in complete Freund's adjuvant and two injections, followed by 2-4 weeks IP immunization (up to a total of 9 immunizations) with the antigen in incomplete Freund's adjuvant. Several dozen mice were immunized for each antigen. A total of 118 mice of the HCo7, HCo12, HCo7+HCo12, and KM strains were immunized with NGF antigen. The immune response was monitored by retroorbital bleeds.

To select HuMab mice producing antibodies that bound human NGF, sera from immunized mice was tested by ELISA as described by Fishwild *et al. supra.*

For preparing cDNA encoding anti-NGF 4D4.D7 kappa light chain, the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA CAT GAG GGT GCC CGC TCA GCT CCT GGG-3'; SEQ ID NO: 36). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sal*I restriction site (5'-CTT GTC GAC TCA ACA CTC TCC CCT GTT GAA GCT C-3'; SEQ ID NO: 37). The resulting PCR product fragment was purified, digested with *Xba*I and *Sal*I, and then gel isolated and ligated into the mammalian expression vector pDSRα20 (*see* International Application, Publication No. WO 90/14363, which is herein incorporated by reference for any purpose. pDSRα20 was produced by changing nucleotide 2563 in pDSRa19 from a "Guanosine" to an "Adenosine" by site directed mutagenesis.).

For preparing cDNA encoding anti- NGF 4D4.D7 heavy chain the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA GTT GGG GCT GTG CTG GGT TTT CCT TGT T-3'; SEQ ID NO: 38). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sal*I restriction site (5'-GCA TGT CGA CTC ATT TAC CCG GAG ACA GGG AGA G-3'; SEQ ID NO: 39). The resulting product was purified, digested with *Xba*I and *Sal*I, gel isolated and ligated into the pDSRα20 vector.

The calculated mass (23099), as determined by translating the nucleotide sequence to predicted amino acids and adding together the molecular weights of the amino acids, of the DNA sequence of the light chain of anti-NGF Ab 4D4 clone matched the measured mass as determined by mass spectrometry. The measured, deconvoluted mass (49479) of the heavy chain of anti-NGF Ab #167 matched the measured mass (49484) of the heavy chain of anti NGF Ab #119 and also matched the theoretical mass (49484) of the DNA sequence of the heavy chain of anti-NGF Ab 4D4 clone (Table 3) within instrumental deviation.

The data of N-terminal protein sequence and LC/MS confirmed that hybridoma #119 expressed the same antibody as hybridoma #167. In addition, the calculated mass of the antibodies based on sequence further confirmed the observation.

We claim:

1. Use of a pharmaceutically effective amount of an NGF antibody for the preparation of a medicament for treating a condition caused by increased expression of nerve growth factor (NGF) or increased sensitivity to NGF comprising administering the NGF antibody to a patient orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional or subcutaneous routes, by sustained release systems or by implantation devices.

2. The use of claim 1, wherein the injection is a subcutaneous injection.

3. The use of claim 2, wherein the pharmaceutically effective amount of the NGF antibody is from about 3 mg to about 30 mg per subcutaneous injection.

4. The use of claim 2, wherein the subcutaneous injection comprises multiple subcutaneous injections.

5. The use of claim 1, wherein the condition is acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine, cluster headache, mixed-vascular or non-vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic neuropathy pain, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or

dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.

6. The use of claim 5, wherein the osteoarthritis is osteoarthritis knee pain.

7. The use of claim 6, wherein the injection is a subcutaneous injection.

8. The use of claim 7, wherein the pharmaceutically effective amount of the NGF antibody is from about 3 mg to about 30 mg per subcutaneous injection.

9. The use of claim 1, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated antibody, wherein the antibody comprises a light chain comprising SEQ ID NO: 44 and a heavy chain comprising SEQ ID NO: 40.

10. The use of claim 9, wherein the heavy chain and light chain of the antibody are connected by a flexible linker to form a single chain antibody.

11. The use of claim 1, wherein the NGF antibody is a Fab' antibody.

12. The use of claim 1, wherein the NGF antibody is a $(Fab')_2$ antibody.

13. The use of claim 1, wherein the NGF antibody is fully human.

14. The use of claim 1, wherein the NGF antibody is humanized.

15. The use of claim 1, wherein the NGF antibody inhibits NGF signaling.

16. The use of claim 1, wherein the NGF antibody comprises a light chain comprising SEQ ID NO: 44 and a heavy chain comprising SEQ ID NO: 40 and the heavy chain and light chain of the antibody are connected by a flexible linker to form a single chain antibody.

17. The use of claim 16, wherein the NGF antibody is a single-chain Fv antibody.

18. The use of claim 16, wherein the NGF antibody is a Fab' antibody.

19. The use of claim 16, wherein the NGF antibody is a $(Fab')_2$ antibody.

20. The use of claim 16, wherein the NGF antibody is fully human.

21. The use of claim 16, wherein the NGF antibody is humanized.

22. The use of claim 16, wherein the NGF antibody inhibits NGF signaling.

23. The use of claim 16, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} or less.

24. The use of claim 16, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-10} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-9} or less.

25. The use of claim 16, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-11} or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 0.2×10^{-9} or less.

Human anti-NGF Neutralizing Antibodies as Selective NGF Pathway Inhibitors

This application claims priority to U.S. patent application Serial No. (U.S.S.N.) 12/576,522, filed October 9, 2009, which is a continuation-in-part application of U.S. patent application Serial No. (U.S.S.N.) 12/277,919, filed November 25, 2008, which is a continuation application of U.S. patent application Serial No. (U.S.S.N.) 10/891,658, filed July 15, 2004, and claims the benefit of priority to U.S. provisional application Serial No. 60/487,431, filed July 15, 2003. This application is also related to U.S. patent application Serial No. 11/767,326, filed June 22, 2007, which is a divisional application of U.S.S.N. 10/891,658.

The sequence listing is filed with the application in electronic format only. The sequence listing text file "02-1240-F-CIP.SeqList.txt" was created on November 25, 2008, and is 79,116 bytes in size.

FIELD OF THE INVENTION

The invention relates to human monoclonal antibodies that bind nerve growth factor (NGF). Compositions and methods for treating pain and pain-related disorders are also described.

BACKGROUND OF THE INVENTION

Every day, more than two million people in the United States are incapacitated by chronic pain (Jessell and Kelly, 1991, "Pain and Analgesia" in PRINCIPLES OF NEURAL SCIENCE, 3rd Ed., (Kandel, Schwartz, and Jessell, ed.), Elsevier, New York). Unfortunately, current treatments for pain are only partially effective, and many of these treatments themselves cause debilitating or dangerous side effects. For example, although non-steroidal anti-inflammatory drugs ("NSAIDs") such as aspirin, ibuprofen, and indomethacin are moderately effective against inflammatory pain, they are also renal toxins, and high doses tend to cause gastrointestinal irritation, ulceration, bleeding, and mental confusion. Patients treated with opioids also frequently experience confusion, and long-term opioid use is associated with

to their use for treating and managing pain, the antibodies of the invention are useful for treating neuropathic and inflammatory pain-related responses.

The invention further provides fusion proteins comprising the sequence of an antibody Fc region and one or more sequences identified as SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NOs: 79-130. Such molecules can be prepared using methods as described, for example, in International Patent Application, Publication No. WO 00/24782. Such molecules can be expressed, for example, in mammalian cells (*e.g.* Chinese Hamster Ovary cells) or bacterial cells (*e.g. E. coli* cells).

In certain aspects, the invention provides antibodies, preferably monoclonal antibodies, most preferably human antibodies and human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof and the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 4.

In certain aspects, the invention provides antibodies, preferably human antibodies, and more preferably monoclonal antibodies, most preferably human monoclonal antibodies, comprising a heavy chain and a light chain, wherein the heavy chain comprises an heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE heavy chain constant regions or any allelic variation thereof (as discussed in Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and the variable region of the heavy chain comprises an amino acid sequence as set forth in SEQ ID NO: 10, or an antigen-binding or an immunologically functional immunoglobulin fragment thereof. Preferably, an antibody of the invention comprises an amino acid sequence of the IgG2 heavy chain constant region as set forth in SEQ ID NO: 4 or an antigen-binding or an immunologically functional immunoglobulin fragment thereof.

Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 11 shows anti-NGF light chain alignment and percent identity for the 14D10 (SEQ ID NO: 82), 6H9 (SEQ ID NO: 84), 7H2 (SEQ ID NO: 86), 4G6a (SEQ ID NO: 88), 4G6b (SEQ ID NO: 89), 4G6c (SEQ ID NO: 90), 4G6d (SEQ ID NO: 91), 4G6e (SEQ ID NO: 131), 14D11 (SEQ ID NO: 80), and 4D4 (SEQ ID NO: 12) antibodies (4G6a is referred to in various Figures as 20031028340; 4G6b is referred to in various Figures as 20031028351; 4G6c is referred to in various Figures as 20031071526; 4G6d is referred to in various Figures as 20031028344; 4G6e is referred to in various Figures as 20031000528).

Figure 12 shows anti-NGF heavy chain alignment and percent identity for the 4D4 (SEQ ID NO: 10), 4G6 (SEQ ID NO: 87), 14D10 (SEQ ID NO: 81), 14D11 (SEQ ID NO: 79), 7H2 (SEQ ID NO: 85), and 6H9 (SEQ ID NO: 83) antibodies.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

Conventional techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.*, 2001, MOLECULAR CLONING: A LABORATORY MANUAL, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Unless specific definitions are provided, the nomenclature utilized in connection with, and

The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. *See, e.g.*, LaPlanche *et al.*, 1986, *Nucl. Acids Res.*, 14:9081; Stec *et al.*, 1984, *J. Am. Chem. Soc.*, 106:6077; Stein *et al.*, 1988, *Nucl. Acids Res.*, 16:3209; Zon *et al.*, 1991, *Anti-Cancer Drug Design*, 6:539; Zon *et al.*, 1991, OLIGONUCLEOTIDES AND ANALOGUES: A PRACTICAL APPROACH, pp. 87-108 (F. Eckstein, Ed.), Oxford University Press, Oxford England; Stec *et al.*, U.S. Pat. No. 5,151,510; Uhlmann and Peyman, 1990, *Chemical Reviews*, 90:543. An oligonucleotide can include a detectable label to enable detection of the oligonucleotide or hybridization thereof.

The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e. g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell. and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The GAP program may be useful with the above parameters. In certain embodiments, the aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

The term "homology" refers to the degree of similarity between protein or nucleic acid sequences. Homology information is useful for the understanding the genetic relatedness of certain protein or nucleic acid species. Homology can be determined by aligning and comparing sequences. Typically, to determine amino acid homology, a protein sequence is compared to a database of known protein sequences. Homologous sequences share common functional identities somewhere along their sequences. A high degree of similarity or identity is usually indicative of homology, although a low degree of similarity or identity does not necessarily indicate lack of homology.

Several approaches can be used to compare amino acids from one sequence to amino acids of another sequence to determine homology. Generally, the approaches fall into two categories: (1) comparison of physical characteristics such as polarity, charge, and Van der Waals volume, to generate a similarity matrix; and (2) comparison of likely substitution of an amino acid in a sequence by any other amino acid, which is based on observation of many protein sequences from known homologous proteins and to generate a Point Accepted Mutation Matrix (PAM).

The percentage of identity may also be calculated by using the program needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 9.0.0 software package, using the default parameters (for example, GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See IMMUNOLOGY--A SYNTHESIS, 2nd Edition, (E. S. Golub and D. R. Gren, Eds.), Sinauer Associates: Sunderland, MA, 1991. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids; unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-

51: 1-84; Ravetch and Bolland, 2001, *Annu. Rev. Immunol.* 19: 275-90; Shields *et al.*, 2001, *Journal of Biol. Chem* 276: 6591-6604; Telleman and Junghans, 2000, *Immunology* 100: 245-251; Medesan *et al.*, 1998, *Eur. J. Immunol.* 28: 2092-2100. Such mutations can include substitutions, additions, deletions, or any combination thereof, and are typically produced by site-directed mutagenesis using one or more mutagenic oligonucleotide(s) according to methods described herein, as well as according to methods known in the art (see, *for example*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 3rd Ed., 2001, Cold Spring Harbor, N.Y. and Berger and Kimmel, METHODS IN ENZYMOLOGY, Volume 152, Guide to Molecular Cloning Techniques, 1987, Academic Press, Inc., San Diego, CA.

According to certain embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (5) confer or modify other physicochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In preferred embodiments, a conservative amino acid substitution typically does not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in PROTEINS, STRUCTURES AND MOLECULAR PRINCIPLES, (Creighton, Ed.), 1984, W. H. Freeman and Company, New York; INTRODUCTION TO PROTEIN STRUCTURE (C. Branden and J. Tooze, eds.), 1991, Garland Publishing, New York, N.Y.; and Thornton *et al.*, 1991, *Nature* 354:105.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". *See*

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

"Antibody" or "antibody peptide(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. In certain embodiments, binding fragments are produced by recombinant DNA techniques. In additional embodiments, binding fragments are produced by enzymatic or chemical cleavage of intact antibodies. Binding fragments include, but are not limited to, F(ab), F(ab'), $F(ab')_2$, Fv, and single-chain antibodies.

The term "heavy chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. The term "light chain" includes any immunoglobulin polypeptide having sufficient variable region sequence to confer specificity for NGF. A full-length heavy chain includes a variable region domain, V_H, and three constant region domains, C_H1, C_H2, and C_H3. The V_H domain is at the amino-terminus of the polypeptide, and the C_H3 domain is at the carboxyl-terminus. The term "heavy chain", as used herein, encompasses a full-length heavy chain and fragments thereof. A full-length light chain includes a variable region domain, V_L, and a constant region domain, C_L. Like the heavy chain,

antibodies or other previously described non-fully human antibodies from non-human species.

One skilled in the art can engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci so that such mice produce human antibodies in the absence of mouse antibodies. Large human Ig fragments may preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse cellular machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains yields high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human MAbs with the desired specificity may be produced and selected.

Transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (see, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551-2555, (1993); Jakobovits et al., Nature, 362:255-258, (1993; Bruggemann et al., Year in Immun., 7:33 (1993); Nature 148:1547-1553 (1994), Nature Biotechnology 14:826 (1996); Gross, J.A., et al., Nature, 404:995-999 (2000); and U.S. patents nos. 5,877,397, 5,874,299, 5,814,318, 5,789,650, 5,770,429, 5,661,016, 5,633,425, 5,625,126, 5,569,825, and 5,545,806). Human antibodies can also be produced in phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1992); Marks et al., J. Mol. Biol., 222:581 (1991)). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therap, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(l):86-95 (1991)).

Recombinant human antibodies may also be 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 those related to human

germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

In certain embodiments, the skilled artisan can use constant regions from species other than human along with the human variable region(s) in such mice to produce chimeric antibodies.

Naturally Occurring Antibody Structure

Naturally occurring antibody structural units typically comprise a tetramer. Each such tetramer typically is composed of two identical pairs of polypeptide chains, each pair having one full-length "light" chain (typically having a molecular weight of about 25 kDa) and one full-length "heavy" chain (typically having a molecular weight of about 50-70 kDa). The amino-terminal portion of each light and heavy chain typically includes a variable region of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant region responsible for effector function. Human light chains are typically classified as kappa and lambda light chains. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM has subclasses including, but not limited to, IgM1 and IgM2. IgA is similarly subdivided into subclasses including, but not limited to, IgA1 and IgA2. Within full-length light and heavy chains, typically, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See, e.g.*, FUNDAMENTAL IMMUNOLOGY, Ch. 7, 2nd ed., (Paul, W., ed.), 1989, Raven Press, N.Y. The variable regions of each light/heavy chain pair typically form the antigen-binding site.

The variable regions typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair typically are aligned by the framework regions, which may enable binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chain

amino acids are specific amino acids that are in positions in which they are not usually found in FRs. The grafted variable regions from anti-NGF antibodies of the invention can be used with a constant region that is different from the constant region of anti-NGF antibody. Alternatively, the grafted variable regions are part of a single chain Fv antibody. CDR grafting is described, *e.g.*, in U.S. Patent Nos. 6,180,370, 5,693,762, 5,693,761, 5,585,089, and 5,530,101.

Antibodies of the invention are preferably prepared using transgenic mice that have a substantial portion of the human antibody producing locus inserted in antibody-producing cells of the mice, and that are further engineered to be deficient in producing endogenous, murine, antibodies. Such mice are capable of producing human immunoglobulin molecules and antibodies and do not produce or produce substantially reduced amounts of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving this result are disclosed in the patents, applications, and references disclosed in the specification herein. In preferred embodiments, the skilled worker may employ methods as disclosed in International Patent Application Publication No. WO 98/24893. *See also* Mendez *et al.*, 1997, *Nature Genetics* 15:146-156.

The monoclonal antibodies (mAbs) of the invention can be produced by a variety of techniques, including conventional monoclonal antibody methodology, *e.g.*, the standard somatic cell hybridization technique of Kohler and Milstein (1975, *Nature* 256:495). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies can be employed, *e.g.*, viral or oncogenic transformation of B-lymphocytes.

The preferred animal system for preparing hybridomas is the mouse. Hybridoma production in the mouse is very well established, and immunization protocols and techniques for isolation of immunized splenocytes for fusion are well known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

In a preferred embodiment, human monoclonal antibodies directed against NGF can be generated using transgenic mice carrying parts of the human immune

system rather than the mouse system. These transgenic mice, referred to herein as "HuMab" mice, contain a human immunoglobulin gene minilocus that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg *et al.*, 1994, *Nature* 368:856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ and in response to immunization, the introduced human heavy chain and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgG κ monoclonal antibodies (Lonberg *et al., supra.*; Lonberg and Huszar, 1995, *Intern. Rev. Immunol.* 13:65-93; Harding and Lonberg, 1995, *Ann. N.Y. Acad. Sci.* 764:536-546). The preparation of HuMab mice is described in detail in Taylor *et al.*, 1992, *Nucleic Acids Res.* 20:6287-6295; Chen *et al.*, 1993, *International Immunology* 5:647-656; Tuaillon *et al.*, 1994, *J. Immunol.* 152:2912-2920; Lonberg *et al.*, 1994, *Nature* 368:856-859; Lonberg, 1994, *Handbook of Exp. Pharmacology* 113:49-101; Taylor *et al.*, 1994, *International Immunology* 6:579-591; Lonberg & Huszar, 1995, *Intern. Rev. Immunol.* 13:65-93; Harding & Lonberg, 1995, *Ann. N.Y. Acad. Sci* 764:536-546; Fishwild *et al.*, 1996, *Nature Biotechnology* 14:845-851. *See further* U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay, as well as U.S. Patent No. 5,545,807 to Surani *et al.*; International Patent Application Publication Nos. WO 93/1227, published June 24, 1993; WO 92/22646, published December 23, 1992; and WO 92/03918, published March 19, 1992. Alternatively, the HCo7, HCo12, and KM transgenic mice strains described in the Examples below can be used to generate human anti-NGF antibodies.

The present invention provides human monoclonal antibodies that are specific for and neutralize bioactive human NGF polypeptides. Also provided are antibody heavy and light chain amino acid sequences which are highly specific for and neutralize NGF polypeptides when they are bound to them. This high specificity enables the anti-human NGF human antibodies, and human monoclonal antibodies with like specificity, to be effective immunotherapy for NGF associated diseases.

In one aspect, the invention provides isolated human antibodies that bind the same or essentially the same epitope as the 4D4 antibody provided herein.

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of anti-NGF antibody, or to increase or decrease the affinity of the anti-NGF antibodies described herein.

As it is well known, minor changes in an amino acid sequence such as deletion, addition or substitution of one, a few or even several amino acids may lead to an allelic form of the original protein which has substantially identical properties. Therefore, in addition to the antibodies specifically described herein, other "substantially homologous" antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. In general, modifications of the genes may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis. Therefore, the present invention contemplates "variant" or "mutant" anti-NGF human antibodies having substantially similar characteristics to the anti-NGF human antibodies disclosed herein (See, for example, WO 00/56772). Thus, by the term "variant" or "mutant" in reference to an anti-NGF human antibody is meant any binding molecule (molecule X) (i) in which the hypervariable regions CDR1, CDR2, and CDR3 of the heavy chain or the hypervariable regions CDR1, CDR2, and CDR3 of the light chain taken as a whole are at least 80% homologous, preferably at least 90% homologous, more preferably at least 95% homologous to the hypervariable regions as shown in SEQ ID NOS: 14, 18, and 22 or SEQ ID NOS: 16, 20, and 24, respectively, and (ii) wherein the variant or mutant is capable of inhibiting the activity of human NGF to the same extent as a reference anti-NGF human antibody having framework regions identical to those of molecule X.

Ordinarily, an anti-NGF human antibody variant will have light and/or heavy chain CDRs, when taken as a whole, that are at least about 80% amino acid sequence identity, preferably at least about 85% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet

The sequences of the 4D4 antibody heavy chain and light chain variable regions are shown in SEQ ID NOS: 10 and 12, respectively. However, many of the potential CDR-contact residues are amenable to substitution by other amino acids and still allow the antibody to retain substantial affinity for the antigen. Likewise, many of the framework residues not in contact with the CDRs in the heavy and light chains can accommodate substitutions of amino acids from the corresponding positions from other human antibodies, by human consensus amino acids, or from other mouse antibodies, without significant loss of the affinity or non-immunogenicity of the human antibody. Selection of various alternative amino acids may be used to produce versions of the disclosed anti-NGF antibodies and fragments thereof that have varying combinations of affinity, specificity, non-immunogenicity, ease of manufacture, and other desirable properties.

In alternative embodiments, antibodies of the invention can be expressed in cell lines other than hybridoma cell lines. In these embodiments, sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. According to these embodiments, transformation can be achieved using any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. Generally, the transformation procedure used may depend upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

A nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of an NGF antibody of the invention is inserted into an appropriate expression vector using standard ligation techniques. In a preferred embodiment, the anti-NGF antibody heavy chain or light chain constant region is appended to the C-terminus of the appropriate variable region and is ligated into an

sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide controlled or sustained release of the product which can be delivered *via* depot injection. In certain embodiments, hyaluronic acid may also be used, having the effect of promoting sustained duration in the circulation. In certain embodiments, implantable drug delivery devices may be used to introduce the desired antibody molecule.

Pharmaceutical compositions of the invention can be formulated for inhalation. In these embodiments, anti-NGF antibodies are advantageously formulated as a dry, inhalable powder. In preferred embodiments, anti-NGF antibody inhalation solutions may also be formulated with a propellant for aerosol delivery. In certain embodiments, solutions may be nebulized. Pulmonary administration and formulation methods therefore are further described in International Patent Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that formulations can be administered orally. Anti-NGF antibodies that are administered in this fashion can be formulated with or without carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In certain embodiments, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the anti-NGF antibody. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

A pharmaceutical composition of the invention is preferably provided to comprise an effective quantity of one or a plurality of anti-NGF antibodies in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions may be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving anti-NGF antibodies in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. *See, for example*, International Patent Application No. PCT/US93/00829, which describes controlled release of porous polymeric microparticles for delivery of pharmaceutical compositions. Sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (as disclosed in U.S. Patent No. 3,773,919 and European Patent Application Publication No. EP 058481, copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-556), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-105), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent Application Publication No. EP 133,988). Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art. See *e.g.*, Eppstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-3692; European Patent Application Publication Nos. EP 036,676; EP 088,046 and EP 143,949.

Pharmaceutical compositions used for *in vivo* administration are typically provided as sterile preparations. Sterilization can be accomplished by filtration through sterile filtration membranes. When the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. Compositions for parenteral administration can be stored in lyophilized form or in a solution. Parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) that is reconstituted prior to administration.

harvested by centrifugation at four hours post induction. Final OD was 75. Expression yield was determined to be approximately 0.15 g/L.

Refolding and purification of rHu-NGF(1-120)

Cell paste was lysed in a Microfluidizer, centrifuged at 10,000 X g for 30 minutes, the pellet was washed with 1% deoxycholic acid, centrifuged as above, and the resulting pellet was then washed with cold water and re-centrifuged. The resulting pellet (WIBs – washed inclusion bodies) was resuspended in denaturant, 8M guanidine HCl, 50mM Tris pH 8.5, containing 10mM DTT, and solubilized at room temperature for 1 hour, centrifuged at 10,000 x g for 30 minutes, and the supernatent was carefully decanted and then diluted 25-fold into an aqueous buffer containing a redox couple at 4°C, for 5 days. The resulting refold was then titrated to pH 3.0, filtered through a 0.45uM filter. The refold was purified using a Sp-Sepharose fast flow column using a standard NaCl gradient. The pool from the cation exchange column was subsequently concentrated and aliquots were frozen –80°C. The purity of the protein was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by Coomassie blue stain. The purified protein was greater than 90% main band by this method.

Example 2

Production of Human Monoclonal Antibodies Against Nerve Growth Factor (NGF)

Transgenic HuMab and KM Mice

Fully human monoclonal antibodies to NGF were prepared using HCo7, HCo12, HCo7+HCo12, and KM strains of transgenic mice, each of which expresses human antibody genes. In each of these mouse strains, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993, *EMBO J.* 12:811-820), and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of International Patent Application Publication No. WO 01/09187. Each of these mouse strains carries a human kappa light chain transgene, KCo5, as described in

Fishwild *et al.* (1996, *Nature Biotechnology* 14:845-851). The HCo7 strain carries the HCo7 human heavy chain transgene as described in U.S. Patent Nos. 5,545,806, 5,625,825, and 5,545,807. The HCo12 strain carries the HCo12 human heavy chain transgene as described in Example 2 of International Patent Application Publication No. WO 01/09187. The HCo7+HCo12 strain carries both the HCo7 and the HCo12 heavy chain transgenes and is hemizygous for each transgene. The KM mice comprises the SC20 heavy chain transgene as described in Tomizuka et al. (1997, *Nature Genet.* 16, 133-143 and 2000, *Proc. Natl. Acad. Sci*, 97, 722-727). This transgene is not integrated into a mouse chromosome, but is instead propagated as an independent chromosome fragment. The fragment includes approximately 15 MB of human chromosome 14. It contains the entire human heavy chain locus including all VH, D and JH gene segments and all heavy chain constant region isotypes. All of these strains are referred to herein as HuMab mice.

HuMab Immunizations:

To generate fully human monoclonal antibodies to NGF, HuMab mice were immunized with purified recombinant NGF derived from *E. coli* cells as antigen (Example 1). General immunization schemes for HuMab mice are described in Lonberg *et al.* (1994, *Nature* 368:856-859; Fishwild *et al.*, *supra.*, and International Patent Application Publication No. WO 98/24884. Mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant preparation (25-100 μg) of NGF antigen was used to immunize the HuMab mice intraperitoneally (IP) or subcutaneously (SC).

Immunizations of HuMab transgenic mice were achieved using antigen in complete Freund's adjuvant and two injections, followed by 2-4 weeks IP immunization (up to a total of 9 immunizations) with the antigen in incomplete Freund's adjuvant. Several dozen mice were immunized for each antigen. A total of 118 mice of the HCo7, HCo12, HCo7+HCo12, and KM strains were immunized with NGF antigen. The immune response was monitored by retroorbital bleeds.

To select HuMab mice producing antibodies that bound human NGF, sera from immunized mice was tested by ELISA as described by Fishwild *et al. supra.*

For preparing cDNA encoding anti-NGF 4D4.D7 kappa light chain, the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA CAT GAG GGT GCC CGC TCA GCT CCT GGG-3'; SEQ ID NO: 36). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sal*I restriction site (5'-CTT GTC GAC TCA ACA CTC TCC CCT GTT GAA GCT C-3'; SEQ ID NO: 37). The resulting PCR product fragment was purified, digested with *Xba*I and *Sal*I, and then gel isolated and ligated into the mammalian expression vector pDSRα20 (*see* International Application, Publication No. WO 90/14363. pDSRα20 was produced by changing nucleotide 2563 in pDSRa19 from a "Guanosine" to an "Adenosine" by site directed mutagenesis.).

For preparing cDNA encoding anti- NGF 4D4.D7 heavy chain the 5' PCR primer encoded the amino terminus of the signal sequence, an *Xba*I restriction enzyme site, and an optimized Kozak sequence (5'-CAG CAG AAG CTT CTA GAC CAC CAT GGA GTT GGG GCT GTG CTG GGT TTT CCT TGT T-3'; SEQ ID NO: 38). The 3' primer encoded the carboxyl terminus and termination codon, as well as a *Sal*I restriction site (5'-GCA TGT CGA CTC ATT TAC CCG GAG ACA GGG AGA G-3'; SEQ ID NO: 39). The resulting product was purified, digested with *Xba*I and *Sal*I, gel isolated and ligated into the pDSRα20 vector.

The calculated mass (23099), as determined by translating the nucleotide sequence to predicted amino acids and adding together the molecular weights of the amino acids, of the DNA sequence of the light chain of anti-NGF Ab 4D4 clone matched the measured mass as determined by mass spectrometry. The measured, deconvoluted mass (49479) of the heavy chain of anti-NGF Ab #167 matched the measured mass (49484) of the heavy chain of anti NGF Ab #119 and also matched the theoretical mass (49484) of the DNA sequence of the heavy chain of anti-NGF Ab 4D4 clone (Table 3) within instrumental deviation.

The data of N-terminal protein sequence and LC/MS confirmed that hybridoma #119 expressed the same antibody as hybridoma #167. In addition, the calculated mass of the antibodies based on sequence further confirmed the observation.

Claims:

1. Use of a pharmaceutically effective amount of an NGF antibody for the preparation of a medicament for treating osteoarthritis (OA) knee pain comprising:

   administering the NGF antibody to a patient orally, through injection by intravenous, intraperitoneal, intracerebral, intra-parenchymal, intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional or subcutaneous routes, by sustained release systems, or by implantation devices.

2. The use of claim 1, wherein the injection is a subcutaneous injection.

3. The use of claim 2, wherein the subcutaneous injection comprises about 3 mg to about 30 mg of the NGF antibody per subcutaneous injection.

4. The use of claim 2 or 3, wherein the subcutaneous injection comprises multiple subcutaneous injections.

5. Use of a pharmaceutically effective amount of an NGF antibody for the preparation of a medicament for treating a condition, comprising:

   administering a pharmaceutically effective amount of an NGF antibody to a patient orally, through injection by intravenous, intraperitoneal, intracerebral, intra-parenchymal, intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, intralesional or subcutaneous routes, by sustained release systems, or by implantation devices;

   wherein the condition is acute pain, dental pain, pain from trauma, surgical pain, pain resulting from amputation or abscess, causalgia, demyelinating diseases, trigeminal neuralgia, cancer, chronic alcoholism, stroke, thalamic pain syndrome, diabetes, acquired immune deficiency syndrome ("AIDS"), toxins, chemotherapy, general headache, migraine, cluster headache, mixed-vascular or non-vascular syndromes, tension headache, general inflammation, arthritis, rheumatic diseases, lupus, osteoarthritis, fibromyalgia, inflammatory bowel disorders, irritable bowel syndrome, inflammatory eye disorders, inflammatory or unstable bladder disorders, psoriasis, skin complaints with inflammatory components, sunburn, carditis, dermatitis, myositis, neuritis, collagen vascular diseases, chronic inflammatory conditions, inflammatory pain and associated hyperalgesia and allodynia, neuropathic pain and associated hyperalgesia or allodynia, diabetic neuropathy pain, sympathetically maintained pain, deafferentation syndromes, asthma, epithelial tissue damage or dysfunction, herpes simplex, disturbances of visceral motility at respiratory, genitourinary, gastrointestinal or vascular regions, wounds, burns, allergic skin reactions, pruritis, vitiligo, general gastrointestinal disorders, colitis, gastric ulceration, duodenal ulcers, vasomotor or allergic rhinitis, or bronchial disorders, dysmenorrhoea, dyspepsia, gastroesophageal reflux, pancreatitis, or visceralgia.

6. The use of claim 5, wherein the injection is a subcutaneous injection.

7. The use of claim 6, wherein the subcutaneous injection comprises about 3 mg to about 30 mg of the NGF antibody per subcutaneous injection.

8. The use of claim 1, comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an isolated antibody, wherein the antibody comprises a light chain comprising SEQ ID NO. 44 and a heavy chain comprising SEQ ID. NO. 40.

9. The use of claim 8, wherein the heavy chain and light chain of the antibody are connected by a flexible linker to form a single chain antibody.

10. The use of claim 1, wherein the NGF antibody is a Fab' antibody.

11. The use of claim 1, wherein the NGF antibody is a $(Fab')_2$ antibody.

12. The use of claim 1, wherein the NGF antibody is fully human.

13. The use of claim 1, wherein the NGF antibody is humanized.

14. The use of claim 1, wherein the NGF antibody inhibits NGF signaling.

15. The use of claim 1, wherein the NGF antibody comprises a light chain comprising SEQ ID NO: 44 and a heavy chain comprising SEQ ID NO:40, and the heavy chain and light chain of the antibody are connected by a flexible linker to form a single chain antibody.

16. The use of claim 15, wherein the NGF antibody is a single-chain Fv antibody.

17. The use of claim 15, wherein the NGF antibody is fully human.

18. The use of claim 15, wherein the NGF antibody inhibits NGF signaling.

19. The use of claim 1 or 5, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-9} M or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-8} M or less.

20. The use of claim 1 or 5, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-10} M or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 1×10^{-9} M or less.

21. The use of claim 1 or 5, wherein the NGF antibody dissociates from a human NGF polypeptide with a K_D of about 1×10^{-11} M or less and neutralizes human NGF bioactivity in a standard *in vitro* assay with an IC_{50} of about 0.2×10^{-9} M or less.