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(54) **Title:**
USE OF SEMAPHORIN 6A FOR PROMOTING MYELINATION AND OLIGODENDROCYTE DIFFERENTIATION

(57) **Abstract:**
-79- ABSTRACT USE OF SEMAPHORIN 6A FOR PROMOTING MYELINATION AND OLIGODENDROCYTE DIFFERENTIATION The invention provides methods of treating diseases, disorsers or injuries involving demyelination and dysmyelination, including multiple sclerosis, by the administration of a Sema6A polypeptide. No Figure

ABSTRACT

USE OF SEMAPHORIN 6A FOR PROMOTING MYELINATION AND OLIGODENDROCYTE DIFFERENTIATION

The invention provides methods of treating diseases, disorsers or injuries involving demyelination and dysmyelination, including multiple sclerosis, by the administration of a Sema6A polypeptide.

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USE OF SEMAPHORIN 6A FOR PROMOTING MYELINATION AND OLIGODENDROCYTE DIFFERENTIATION

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates to neurobiology, neurology and pharmacology. More particularly, it relates to methods of treating diseases relating to central nervous system myelination by the administration of semaphorin 6A ("Sema6A") polypeptide.

Background Art

[0002] Many diseases of the nervous system are associated with demyelination and dysmyelination, including multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), Wallerian Degeneration and some inherited diseases such as adrenoleukodystrophy, Alexander's disease, and Pelizaeus Merzbacher disease (PMZ). Among these diseases, MS is the most widespread, affecting approximately 2.5 million people worldwide.

[0003] MS generally begins with a relapsing-remitting pattern of neurologic involvement, which then progresses to a chronic phase with increasing neurological damage. MS is associated with the destruction of myelin, oligodendrocytes and axons localized to chronic lesions. The demyelination observed in MS is not always permanent and remyelination has been documented in early stages of the disease. Remyelination of central nervous system ("CNS") neurons requires oligodendrocytes.

[0004] Various disease-modifying treatments are available for MS, including the use of corticosteroids and immunomodulators such as interferon beta. In addition, because of the central role of oligodendrocytes and myelination in MS, there have been efforts to develop therapies to increase oligodendrocyte numbers or enhance myelination. *See, e.g.*, Cohen *et al.*, U.S. Pat. No. 5,574,009; Chang *et al., N. Engl. J. Med. 346*:165-73 (2002). However, there remains an urgent need to devise additional therapies for MS.

[0005] Semaphorins are secreted or membrane-bound proteins that are known to control axon guidance and cell migration. Kerjan *et al., Nat. Neursci.* 8(11): 1516-1524 (2005). Many transmembrane semaphorins are expressed in the developing CNS, but little is

known of their functions *in vivo*. *Id.* Class 6 semaphorins comprise four proteins, Sema6A-Sema6D, that are closely related to invertebrate transmembrane semaphorins. Fiore & Puschel. *Front. Biosci.* 8:2484-2499 (2003). All semaphorins possess a semaphorin (Sema) domain and a plexin-semaphorin-integrin (PSI) domain (found in plexins, semaphorins and integrins) in the N-terminal extracellular portion.

[0006] Plexins are a family of molecules (the plexin family) which are distributed in various animal species. Murakami *et al.*, *Dev. Dynam.* 220: 246-258 (2001). Plexins are grouped into four sub-families, *i.e.*, plexin-A, -B, -C, and -D. *Id.* In mouse and human, four members of the plexin-A subfamily (plexin-A1, -A2, -A3, and -A4) have been isolated. *See* Kameyama *et al.*, *Biochem. Biophys. Res. Commun.* 226: 396 - 402 (1996); Kameyama *et al.*, *Biochem. Biophys. Res. Commun.* 226: 524 - 529 (1996); Maestrini *et al.*, *Proc. Natl. Acad. Sci. USA* 93: 674 - 678 (1996); Tamagnone *et al.*, *Cell* 99: 71 - 80 (1999); and Suto *et al.*, *Mech. Dev.* 120: 385 - 396 (2003). The ectodomains of the plexin-A subfamily possess a stretch of approximately 500 amino acids (aa) residues which exhibit significant homology to the sema domain shared by semaphorins. Murakami *et al.*, *Develop. Dyn.* 220: 246-258 (2001). Type-A plexins are known to interact with class 6 semaphorins. Toyofuku *et al.*, *Genes Develop.* 18: 435-447 (2004). For example, Suto *et al.* showed that plexin-A4 is a direct receptor for Sema6A. *J. Neurosci.* 25(14): 3628-3637 (2005).

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention is based on the discovery that semaphorin 6A (Sema6A) is expressed in oligodendrocytes and regulates oligodendrocyte differentiation, survival and/or axon myelination. Furthermore, certain Sema6A polypeptides promote survival, proliferation and/or differentiation of oligodendrocytes as well as myelination of neurons. Based on these discoveries, the invention relates generally to methods of treating conditions associated with demyelination and/or dysmyelination (*e.g.* multiple sclerosis) by the administration of a Sema6A polypeptide.

[0008] In certain embodiments, the invention includes a method for promoting proliferation, differentiation, or survival of oligodendrocytes, comprising contacting oligodendrocytes with an effective amount of a composition comprising an isolated Sema6A polypeptide. In other embodiments, the invention includes a method for

promoting oligodendrocyte-mediated myelination of neurons, comprising contacting a mixture of neurons and oligodendrocytes with an effective amount of a composition comprising a Sema6A polypeptide.

[0009] The present invention is directed to a method for promoting proliferation, differentiation, or survival of oligodendrocytes in a mammal or a method for promoting myelination of neurons in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising a Sema6A polypeptide.

[0010] Also included is a method for treating a disease, disorder, or injury associated with dysmyelination or demyelination or associated with oligodendrocyte death or lack of differentiation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising a Sema6A polypeptide.

[0011] Further included is a method for treating a disease, disorder, or injury involving the destruction of myelin in a mammal comprising administering a therapeutically effective amount of a composition comprising a Sema6A polypeptide.

[0012] Additionally included is a method of the present invention described herein, where the Sema6A polypeptide binds to a plexin-A2 polypeptide or a plexin-A4 polypeptide. In other embodiments, the Sema6A polypeptide is isolated.

[0013] Further embodiments of the invention include a method of treating a disease, disorder or injury involving the destruction of oligodendrocytes or myelin by *in vivo* gene therapy, comprising administering to a mammal, at or near the site of the disease, disorder or injury, a vector comprising a nucleotide sequence that encodes a Sema6A polypeptide so that the Sema6A polypeptide is expressed from the nucleotide sequence in the mammal in an amount sufficient to promote axonal extension by neurons at or near the site of the injury. In certain embodiments, the present invention includes a method for promoting proliferation, differentiation, or survival of oligodendrocytes or for promoting myelination of neurons in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising a polynucleotide, which encodes a Sema6A polypeptide.

[0014] Additionally, the invention includes a method for treating a disease, disorder, or injury associated with dysmyelination or demyelination or associated with oligodendrocyte death or lack of differentiation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising

a polynucleotide, which encodes a Sema6A polypeptide. The invention further includes a method for treating a disease, disorder, or injury involving the destruction of myelin in a mammal comprising administering a therapeutically effective amount of a composition comprising a polynucleotide, which encodes a Sema6A polypeptide. In certain embodiments, the Sema6A polypeptide of the present invention binds to a plexin-A2 polypeptide or a plexin-A4 polypeptide. The polynucleotide used in the method of the present invention can be isolated.

[0015] In certain embodiments, the vector is a viral vector which is selected from the group consisting of an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector, an Epstein Barr viral vector, a papovaviral vector, a poxvirus vector, a vaccinia viral vector, and a herpes simplex viral vector.

[0016] In some embodiments, the disease, disorder, injury or condition is selected from the group consisting of multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), adrenoleukodystrophy, Alexander's disease, Pelizaeus Merzbacher disease (PMZ), Globoid cell Leucodystrophy (Krabbe's disease), Wallerian Degeneration, optic neuritis, transverse myelitis, amylotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, spinal cord injury, traumatic brain injury, post radiation injury, neurologic complications of chemotherapy, stroke, acute ischemic optic neuropathy, vitamin E deficiency, isolated vitamin E deficiency syndrome, AR, Bassen-Kornzweig syndrome, Marchiafava-Bignami syndrome, metachromatic leukodystrophy, trigeminal neuralgia, and Bell's palsy. In some embodiments, the cultured host cell is derived from the mammal to be treated.

[0017] Certain Sema6A polypeptides include, but are not limited to, Sema6A polypeptides fragments, variants, or derivatives thereof which lack a transmembrane domain and a cytoplasmic domain. Sema6A polypeptides include polypeptides comprising (i) a signal sequence, (ii) a sema domain, (iii) a PSI domain, (iv) an extracellular domain, (v) a transmembrane domain, (vi) a cytoplasmic domain, and (vii) a combination of two or more of the domains. In some embodiments, the Sema6A polypeptide lacks a signal sequence, a sema domain, a PSI domain, a transmembrane domain, a cytoplasmic domain, or a combination of two or more of the domains. In some

embodiments, the Sema6A polypeptide comprises a Sema domain and lacks a signal sequence, a PSI sequence, a transmembrane domain, and a cytoplasmic domain. In some embodiments, the Sema6A polypeptide comprises amino acid residues 1-649 of SEQ ID NO: 2.

[0018] In some embodiments, the Sema6A polypeptide is administered by bolus injection or chronic infusion. In some embodiments, the Sema6A polypeptide is administered directly into the central nervous system. In some embodiments, the Sema6A polypeptide is administered directly into a chronic lesion of MS.

[0019] In some embodiments, the Sema6A polypeptide is a fusion polypeptide comprising a non-Sema6A moiety. In some embodiments, the non-Sema6A moiety is selected from the group consisting of an antibody Ig moiety, a serum albumin moiety, a targeting moiety, a reporter moiety, and a purification-facilitating moiety. In some embodiments, the antibody Ig moiety is a hinge and Fc moiety.

[0020] In some embodiments, the polypeptides of the present invention are conjugated to a polymer. In some embodiments, the polymer is selected from the group consisting of a polyalkylene glycol, a sugar polymer, and a polypeptide. In some embodiments, the polyalkylene glycol is polyethylene glycol (PEG). In some embodiments, the polypeptides of the present invention are conjugated to 1, 2, 3 or 4 polymers. In some embodiments, the total molecular weight of the polymers is from 5,000 Da to 100,000 Da.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0021] Figure 1 is a sequence comparison among human Sema6A polypeptide isoforms, *i.e.*, isoforms A-D.

[0022] Figure 2 is a sequence comparison among mouse Sema6A polypeptide isoforms, *i.e.*, isoforms 1-3.

[0023] Figures 3: Sema6A expression in mouse nervous system. Sema6A expression of in the P15 mouse nervous system was analyzed by *In situ* hybridization. Most cells expressing Sema6A are found in the white matter. Sema6A expressing cells in the white matter are oligodendrocytes.

[0024] Figure 4A-4C: Immunostaining analysis of Myelin proteolipid protein (PLP) expressing Sema6A +/- (A) or Sema6A -/- (B) oligodendrocytes in the anterior

commissure (AC) at P16. (C) - Quantification of PLP expressing cells in AC at different stages, *i.e.*, P16, P30, and P45.

[0025] Figure 5A-5D: (A) - *In vitro* maturation of Sema6A -/- oligodendrocytes. The fractal dimension (FD) of the oligodendrocytes was measured after 48 hrs. Left bars are FD of Sema6A -/- oligodendrocytes, and right bars are FD of Sema6A +/- oligodendrocytes. (B) - Oligodendrocytes of Sema6A -/- at 48 hrs visualized by phase contrast microscopy and immunostaining with anti-O4 antibody. (C): *In vitro* maturation of Sema6A -/- oligodendrocyte at 24 hr and 48 hr. Left bars are 24 hr, and right bars are 48 hr. (D): Oligodendrocytes of Sema6A+/- at 48 hrs visualized by phase contrast microscopy and immunostaining with anti-O4 antibody.

[0026] Figures 6A-6C: Myelination in cocultures of the dorsal root ganglion cells (DRG) and oligodendrocytes with addition of Sema6A-Fc at various dosages, (A) negative control; (B) 0.1μg/ml; and (C) 0.3μg/ml. The degree of myelination is shown by immunostaining with anti MBP antibody.

[0027] Figure 7: Mice were treated with cuprizone and examined for the Sema6A expression during demyelination and remyelination. The number of Sema6A expressing cells was measured at different stages, *i.e.*, 3-6 weeks.

[0028] Figure 8: (A)-(B) *In situ* hybridization using a Sema6A riboprobe in human MS lesion tissue and non-lesion tissue at x1 magnification (A) and x1 magnification(B); (C) Immunostaining of human MS lesion tissue using human Sema6A antibody at x10 magnification.

[0029] Figure 9A-D: (A) Western blot analysis of plexin-A2 polypeptide expression in plexin-A2 +/+ (wildtype), plexin-A2 protein null knockout (plexin-A2-/-) mice and plexin-A2 mutant bearing a single amino acid substitution (A396E) in the Semaphorin domain (NMF454); (B) Sequence alignment of plexin-A2, plexin-A4, plexin-A1, and plexin-A3 to identify alanine (396); (C) Sema6A binding assay to wildtype plexin-A2 protein expressed in COS cells; and (D) Sema6A binding assay to mutated plexin-A2A396E expressed in COS cells.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0031] Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

[0032] In order to further define this invention, the following terms and definitions are provided.

[0033] It is to be noted that the term "a" or "an" entity, refers to one or more of that entity; for example, "a polypeptide," is understood to represent one or more polypeptides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0034] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," indicate the inclusion of any recited integer or group of integers but not the exclusion of any other integer or group of integers.

[0035] Throughout the specification and claims, the term "consists of" and variations such as "consist of" or "consisting of" indicate the inclusion of any recited integer or group of integers but that no additional integer or group of integers may be added to the specified method, structure, or composition.

[0036] Throughout the specification and claims, the term "consists essentially of" and variations such as "consist essentially of" or "consisting essentially of" indicate the inclusion of any recited integer or group of integers and the optional inclusion of any

recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure, or composition.

[0037] As used herein, "antibody" means an intact immunoglobulin, or an antigen-binding fragment thereof. Antibodies of this invention can be of any isotype or class (*e.g.*, M, D, G, E and A) or any subclass (*e.g.*, G1-4, A1-2) and can have either a kappa (κ) or lambda (λ) light chain.

[0038] As used herein, "Fc" means a portion of an immunoglobulin heavy chain that comprises one or more heavy chain constant region domains, CH1, CH2 and CH3. For example, a portion of the heavy chain constant region of an antibody that is obtainable by papain digestion.

[0039] As used herein, "humanized antibody" means an antibody in which at least a portion of the non-human sequences are replaced with human sequences. Examples of how to make humanized antibodies may be found in United States Patent Nos. 6,054,297, 5,886,152 and 5,877,293.

[0040] As used herein, "chimeric antibody" means an antibody that contains one or more regions from a first antibody and one or more regions from at least one other antibody. The first antibody and the additional antibodies can be from the same or different species.

[0041] As used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result may be, *e.g.*, lessening of symptoms, prolonged survival, improved mobility, and the like. A therapeutic result need not be a "cure".

[0042] As used herein, a "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0043] As used herein, a "polynucleotide" can contain the nucleotide sequence of the full length cDNA sequence, including the untranslated 5' and 3' sequences, the coding sequences, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. The polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded

DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. Polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0044] In the present invention, a polypeptide can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids (*e.g.* non-naturally occurring amino acids). The polypeptides of the present invention may be modified by either natural process, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation,

prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (*See*, for instance, Proteins - Structure And Molecular Properties, 2nd Ed., T.E. Creighton, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol* 182:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci* 663:48-62 (1992).)

[0045] The terms "fragment," "variant," "derivative" and "analog" when referring to a Sema6A polypeptide of the present invention include any polypeptides which retain at least some immunogenicity, *i.e.*, the ability to induce an immune response against sema6A, or any naturally-occurring function of Sema6A, *e.g.*, the ability to bind to any one of plexin-A subfamily polypeptides, *i.e.*, plexin-A1, plexin-A2, plexin-A3, or plexin-A4. An example of the naturally-occurring Sema6A function is its ability to bind to plexin-A2 or plexin-A4 polypeptide. Sema6A polypeptides as described herein may include fragment, variant, or derivative molecules without limitation, so long as the Sema6A polypeptide still retains immunogenicity or any one naturally-occuring function. Sema6A polypeptides of the present invention may include Sema6A proteolytic fragments, deletion fragments and in particular, fragments which more easily reach the site of action when delivered to an animal. Polypeptide fragments further include any portion of the polypeptide which comprises an antigenic or immunogenic epitope of the native polypeptide, including linear as well as three-dimensional epitopes. Sema6A polypeptides of the present invention may comprise variant Sema6A regions, including fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally, such as an allelic variant. By an "allelic variant" is intended alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Sema6A polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions. Sema6A polypeptide of the present invention may also include derivative molecules. For example, Sema6A polypeptides of the present invention may include Sema6A regions which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins and protein conjugates.

[0046] In the present invention, a "polypeptide fragment" or "protein fragment" refers to a short amino acid sequence of a Sema6A polypeptide. Protein or polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part of region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising about 5 amino acids, about 10 amino acids, about 15 amino acids, about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, and about 100 amino acids.

[0047] As used herein, the terms "linked," "fused" or "fusion" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single protein containing two ore more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments may be physically or spatially separated by, for example, in-frame linker sequence.

[0048] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

[0049] The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, an RNA or polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product and the translation of such mRNA into polypeptide(s). If the final desired product is biochemical, expression includes the creation of that biochemical and any precursors.

[0050] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans, domestic animals, farm animals, zoo animals, sport animals, pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; bears, food animals such as cows, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. In certain embodiments, the mammal is a human subject.

Sema6A

[0051] The invention is based on the discovery that Sema6A polypeptides increase oligodendrocyte numbers by promoting their survival, proliferation and/or differentiation. In addition, the inventors have discovered that Sema6A polypeptides promote myelination of neurons.

[0052] Naturally occurring human Sema6A polypeptide is known to be expressed in developing brain, kidney, lung and liver. Sema6A is also detected in human adult tissues such as the skin (dendritic cells), and in the highly regenerative placental tissues. The human Sema6A gene consists of 20 exons, including 2 untranslated exons, covering approximately 60 kb of genomic sequence on chromosome 5.

[0053] The full-length human Sema6A polypeptide consists of a signal sequence, an extracellular domain, a transmembrane domain and a cytoplasmic domain. The extracellular domain comprises a sema domain and a plexin-semaphorin-integrin (PSI) domain. Full-length human Sema6A polypeptides vary from about 971 amino acids to about 1049 amino acids in length, depending on the variants. *See* Figure 1. Similar variants occur in mouse Sema6A. *See, e.g.*, Figure 2.

[0054] A polypeptide sequence of 1030aa was reported as a human Sema6A polypeptide sequence and has the accession number NP-065847 in Genbank. The human Sema6A polypeptide sequence is designated herein as isoform A and SEQ ID NO: 2. SEQ ID NO: 1 is a nucleotide sequence encoding SEQ ID NO: 2. A polypeptide sequence of 1047aa was reported as a variant of the human Sema6A polypeptide sequence and has the accession number EAW48937 in Genbank. The 1047aa polypeptide is desginated herein

as isoform B and SEQ ID NO: 4. A nucleotide sequence encoding SEQ ID NO: 4 is SEQ ID NO: 3. Another variant of the human Sema6A polypeptide having 971aa was reported as the accession number EAW48935 in Genbank. The 971aa polypeptide is desginated herein as isoform C and SEQ ID NO: 6. A polypeptide sequence of 975aa was reported as a human Sema6A polypeptide variant and has the accession number EAW48934 in Genbank. The 975aa polypeptide sequence is desginated herein as isoform D and SEQ ID NO: 8. Variants of human Sema6A include, but are not limited to, the Sema6A polypeptide of isoform A with a deletion after amino acids 1001, resulting in a polypeptide with 1000 amino acids. Nakayama *et al.*, *Genome Res.* 12(11): 1773–1784 (2002); Strausberg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 99(26): 16899-16903 (2002). Other Sema6A variants are also known, for example, in Prislei *et al. Mol Cancer Ther.* 7(1): 233-241 (2007).

[0055] The mouse Sema6A polypeptide sequence and its variants are also reported. The 1031 aa mouse Sema6A polypeptide has the accession number O35464 in UniProtKB/Swiss-Prot entry. The polypeptide is desginated herein as isoform 1 and SEQ ID NO: 10. A nucleotide sequence encoding SEQ ID NO: 10 is desginated as SEQ ID NO: 9. Another polypeptide sequence of 1005aa was reported as a mouse Sema6A polypeptide sequence and has the accession number AF288666 in Genbank. The polypeptide sequence is designated as isoform 2 and SEQ ID NO: 12. A nucleotide sequence encoding SEQ ID NO: 12 is desginated herein as SEQ ID NO: 11. Another variant of the mouse Sema6A polypeptide sequence was reported as the accession number O35464 in UniProtKB/Swiss-Prot entry. The polypeptide sequence is designated herein as isoform 3 and SEQ ID NO: 14. A nucleotide sequence encoding SEQ ID NO: 14 is SEQ ID NO: 13. Variants of mouse Sema6A include, but are not limited to, the polypeptides with the following mutations: A172V, L201P, N337D, S585N, Q685R, TK703-704SE, P735S, Q766E, I856T, or KSPNHGVNLVENLDSLPPKVPQREAS863-888ESSPYVLKQFSEAFNRQGHLSVAVE.

[0056] Sema6A polypeptides known in other animals include, but are not limited to, chimpanzee, dog, and zebra fish. There are variants of Chimpanzee Sema6A polypeptides, *e.g.*, Genbank Accession Nos. XP_001150634, XP_001150901, XP_001150706, XP_001151177, XP_001151109, XP_001151041, XP_001150971, and XP_517889. Non-limiting examples of dog Sema6A polypeptide variants are Genbank

Accession Nos. XP_538552, XP_859002, XP_858964, XP_858921, XP_858886, and XP_858843. Sema6A polypeptides and the variants can be found in other animals.

[0057] The Sema6A functional domain designations may be defined as follows:

TABLE 1. Example Sema6A domains for human.

Domain	Sema6A (isoform A) 1030aa	Sema6A (isoform B) 1047aa	Sema6A (isoform C) 971aa	Sema6A (isoform D) 975aa
Signal Seq.	1-18	1-18	1-18	1-18
Sema Domain	56-472	56-472	56-418	56-472
PSI Domain	514-551	514-551	456-492	514-551
Transmembrane	650-670	667-687	591-611	595-615
Cytoplasmic	671-1030	688-1047	612-971	616-975

TABLE 2. Example Sema6A domains for mice.

Domain	Sema6A (isoform 1) 1031aa	Sema6A (isoform 2) 1005aa	Sema6A (isoform 3) 976aa
Signal Seq.	1-18	1-18	1-18
Sema Domain	56-474	56-448	56-474
PSI Domain	514-547	488-521	514-547
Transmembrane	650-670	624-644	595-615
Cytoplasmic	671-1031	645-1005	616-976

[0058] As one of skill in the art will appreciate, the beginning and ending residues of the domains listed above may vary depending upon the computer modeling program used or the method used for determining the domain. As such, various functional domains of Sema6A may vary from those defined above. For example, the functional domains of human Sema6A polypeptide isoform A, *i.e.*, SEQ ID NO: 2, can vary as follows:

TABLE 3. Sequence Variations of Functional Domains of SEQ ID NO: 2.

	Signal Seq.	Sema Domain	PSI Domain	Transmembrane domain	Cytoplasmic domain	Low complexity Region
SMART	1-18	56-487	514-569	648-670	671-1030	937-952
PROSITE	n/a	24-512	n/a	n/a	n/a	n/a
pFam	n/a	56-491	514-565	n/a	n/a	n/a
UniPort/ Swiss Port	1-18	24-512	n/a	650-670	671-1030	n/a
NCBI (NP 065847)	n/a	56-472	514-551	n/a	n/a	n/a
Klostermann et al.	5-20	42-564	n/a	648-671	671-1030	n/a

[0059] Based on the sequence variation in SEQ ID NO: 2, a person of ordinary skill in the art can identify sequence variations in SEQ ID NOs: 4, 6, and 8.

[0060] The sequences of the functional domains in SEQ ID NO: 10, *i.e.*, isoform 1 of the mouse Sema6A polypeptide sequence, vary as follows:

TABLE 4. Sequence Variations of Functional Domains of SEQ ID NO: 10

	Signal Seq.	Sema Domain	PSI Domain	Transmembrane domain	Cytoplasmic domain	Low complexity Region
SMART	1-18	56-487	514-569	648-670	671-1031	937-951
PROSITE	n/a	24-512	n/a	n/a	n/a	n/a
pFam	n/a	56-491	514-569	n/a	n/a	n/a
UniProtKB/ Swiss Prot	1-18	24-512	n/a	650-670	671-1031	n/a
NCBI (NP 061214)	n/a	56-474	514-547	n/a	n/a	n/a

[0061] In addition, the sequence variations in SEQ ID NOs: 12 or 14 or any other variants in mouse or other animals can readily be identified based on Tables 2 - 4.

Plexin-A2

[0062] The plexin-A2 polypeptide is known to bind to Sema6A polypeptide. Suto *et al. Neuron* 53: 535 (2007). The full-length human plexin-A2 polypeptide (SEQ ID NO: 15) consists of a signal sequence, an extracellular domain, a transmembrane domain and a cytoplasmic domain. The extracellular domain comprises a sema domain and four IPT/TIG domains, *i.e.*, IPT/TIGs 1-4. The sema domain is amino acids 50-523 of SEQ ID NO: 15. The IPT/TIG domains 1-4 are amino acids 873-967, 967-1053, 1056-1155, and 1158-1251 of SEQ ID NO: 15, respectively. As one of skill in the art will appreciate, the beginning and ending residues of the domains listed above may vary depending upon the computer modeling program used or the method used for determining the domain.

[0063] The sequences of full-length human plexin-A2 polypeptides vary. One example of a plexin-A2 polypeptide variant has 1894 aa sequence and has the accession number NP 079455 in Genbank. Also, plexin-A2 sequences from other animals are well known in the art. For example, mouse plexin-A2 polypeptides are known in the art and reported as NP_032908, AAH68155, EDL12938, EDL12937, and NP_786926 in Genbank.

Plexin-A4

[0064] Plexin-A4 is also known to be a receptor of Sema6A polypeptide. Suto *et al. Neuron* 53: 535 (2007). The full length human plexin-A4 polypeptide (SEQ ID NO: 16) consists of a signal sequence, an extracellular domain, a transmembrane domain and a cytoplasmic domain. The extracellular domain comprises a sema domain, three PSI domains, *i.e.*, PSIs 1-3, and four IPT/TIG domains, *i.e.*, IPT/TIGs 1-4. The sema domain is amino acids 24-507 of SEQ ID NO: 16. PSI domains 1-3 are amino acids 509-559, 655-702, and 803-856 of SEQ ID NO: 16, respectively. IPT/TIG domains 1-4 of the plexin-A4 polypeptide are amino acids 858-952, 954-1037, 1040-1139, and 1142-1230 of SEQ ID NO: 16, respectively. An artisan appreciates that the beginning and ending residues of the domains listed above may vary depending upon the computer modeling program used or the method used for determining the domain.

[0065] Sequences of full-length human plexin-A4 polypeptides vary. For example, several variants of plexin-A4 are reported as EAW83796, NP_001099013, EAW83795, AAH28744, and EAL24077 in Genbank. Furthermore, plexin-A4 sequences from other

animals are also reported. For example, mouse plexin-A4 polypeptides are known as NP_786926, BAC56599, EDL13705, and EDL13704 in Genbank.

[0066] Some embodiments of the invention provide a full-length or mature Sema6A polypeptide or a soluble Sema6A polypeptide. Specifically, soluble Sema6A polypeptides of the present invention include fragments, variants, or derivatives thereof of a full-length or mature Sema6A polypeptide. Tables 1-4 above describe the various functional domains of the Sema6A polypeptide. Soluble Sema6A polypeptides of the invention generally comprise a portion or all of the extracellular domain of the polypeptides. Soluble Sema6A polypeptides generally lack the transmembrane domain and/or cytoplasmic domains. As one of skill in the art would appreciate, the entire extracellular domain of Sema6A may comprise additional or fewer amino acids on either the C-terminal or N-terminal end of the extracellular domain polypeptide, and may contain internal deletions.

[0067] Human Sema6A polypeptides for use in the methods of the present invention include, but are not limited to, a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein said reference amino acid sequence is selected from the group consisting of amino acids 56 to 417 of SEQ ID NO: 2; a to 417 of SEQ ID NO:2; b to 417 of SEQ ID NO:2; 1 to 417 of SEQ ID NO:2; 56 to c of SEQ ID NO: 2; a to c of SEQ ID NO: 2; b to c of SEQ ID NO: 2; 1 to c of SEQ ID NO: 2; 56 to c' of SEQ ID NO: 6; a to c' of SEQ ID NO: 6; b to c' of SEQ ID NO: 6; 1 to c' of SEQ ID NO: 6; 56 to d of SEQ ID NO: 2; a to d of SEQ ID NO: 2; b to d of SEQ ID NO: 2; 1 to d of SEQ ID NO: 2; 56 to d' of SEQ ID NO: 6; a to d' of SEQ ID NO: 6; b to d' of SEQ ID NO: 6; 1 to d' of SEQ ID NO: 6; 56 to e of SEQ ID NO: 2; a to e of SEQ ID NO: 2; b to e of SEQ ID NO: 2; 1 to e of SEQ ID NO: 2; 56 to e' of SEQ ID NO: 6; a to e' of SEQ ID NO: 6; b to e' of SEQ ID NO: 6; 1 to e' of SEQ ID NO: 6; 56 to e" of SEQ ID NO: 8; a to e" of SEQ ID NO: 8; b to e" of SEQ ID NO: 8; 1 to e" of SEQ ID NO: 8; 56 to e"' of SEQ ID NO: 4; a to e"' of SEQ ID NO: 4; b to e"' of SEQ ID NO: 6; 1 to e"' of SEQ ID NO: 8; 56 to f of SEQ ID NO: 2; a to f of SEQ ID NO: 2; b to f of SEQ ID NO: 2; 1 to f of SEQ ID NO: 2; 56 to f' of SEQ ID NO: 6; a to f' of SEQ ID NO: 6; b to f' of SEQ ID NO: 6; 1 to f' of SEQ ID NO: 6; 56 to f" of SEQ ID NO: 8; a to f" of SEQ ID NO: 8; b to f" of SEQ ID NO: 8; 1 to f" of SEQ ID NO: 8; 56 to f"' of SEQ ID NO: 4;

a to f''' of SEQ ID NO: 4; b to f''' of SEQ ID NO: 4; 1 to f''' of SEQ ID NO: 4; and a combination of two or more of said amino acid sequences; wherein a is any integer between 24 and 56, b is any integer between 19 and 21, c is any integer between 472 and 512, c' is any integer between 418 and 453, d is any integer between 514 and 569, d' is any integer between 455 and 510, e is any integer between 570 and 650, e' is any integer between 511 and 591, e'' is any integer between 570 and 595, and e''' is any integer between 570 and 667; f is any integer between 647 and 671, f' is any integer between 588 and 612, f'' is any integer between 592-616, and f''' is any integer between 664 and 688. In certain embodiments, the Sema6A polypeptide for use in the methods of the present invention binds to a plexin-A2 or to a plexin-A4 polypeptide.

[0068] In certain embodiments, human Sema6A polypeptides for use in the methods of the present invention include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids 1 to 975 of SEQ ID NO: 8; 19 to 417 of SEQ ID NO: 2; 19 to 472 of SEQ ID NO: 2; 19 to 551 of SEQ ID NO: 2; 19 to 492 of SEQ ID NO: 6; 19 to 647 of SEQ ID NO:2; 19 to 588 of SEQ ID NO: 6; 19 to 592 of SEQ ID NO: 8; 19 to 664 of SEQ ID NO: 4; 56 to 472 of SEQ ID NO: 2; 56 to 551 of SEQ ID NO: 2; 56 to 492 of SEQ ID NO: 6; 56 to 647 of SEQ ID NO:2; 56 to 588 of SEQ ID NO: 6; 56 to 592 of SEQ ID NO: 8; 56 to 664 of SEQ ID NO: 4; 1 to 649 of SEQ ID NO: 2; [human Sema6A-Fc from R&D] 1 to 590 of SEQ ID NO: 6; 1 to 594 of SEQ ID NO: 8; 1 to 666 of SEQ ID NO: 4; 18 to 703 of SEQ ID NO: 2; 18 to 644 of SEQ ID NO: 6; 18 to 648 of SEQ ID NO: 8; 18 to 720 of SEQ ID NO: 4; 1 to 648 of SEQ ID NO: 2; 1 to 589 of SEQ ID NO: 6; 1 to 593 of SEQ ID NO: 8; 1 to 665 of SEQ ID NO: 4; and a combination of two or more of said amino acid sequences. In another embotidments, the Sema6A polypeptide binds to plexin-A2 or plexin-A4 polypeptides.

[0069] In another embodiment, human Sema6A polypeptides for use in the methods of the present invention include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids 56-417 of SEQ ID NO: 2; 55-417 of SEQ ID NO: 2; 54-417 of SEQ ID NO: 2; 53-417 of SEQ ID NO: 2; 52-417 of

SEQ ID NO: 2; 51-417 of SEQ ID NO: 2; 50-417 of SEQ ID NO: 2; 49-417 of SEQ ID NO: 2; 48-417 of SEQ ID NO: 2; 47-417 of SEQ ID NO: 2; 46-417 of SEQ ID NO: 2; 45-417 of SEQ ID NO: 2; 44-417 of SEQ ID NO: 2; 43-417 of SEQ ID NO: 2; 42-417 of SEQ ID NO: 2; 41-417 of SEQ ID NO: 2; 40-417 of SEQ ID NO: 2; 39-417 of SEQ ID NO: 2; 38-417 of SEQ ID NO: 2; 37-417 of SEQ ID NO: 2; 36-417 of SEQ ID NO: 2; 35-417 of SEQ ID NO: 2; 34-417 of SEQ ID NO: 2; 33-417 of SEQ ID NO: 2; 32-417 of SEQ ID NO: 2; 31-417 of SEQ ID NO: 2; 30-417 of SEQ ID NO: 2; 29-417 of SEQ ID NO: 2; 28-417 of SEQ ID NO: 2; 27-417 of SEQ ID NO: 2; 26-417 of SEQ ID NO: 2; 25-417 of SEQ ID NO: 2; 24-417 of SEQ ID NO: 2; 23-417 of SEQ ID NO: 2; 22-417 of SEQ ID NO: 2; 21-417 of SEQ ID NO: 2; 20-417 of SEQ ID NO: 2; 19-417 of SEQ ID NO: 2; 18-417 of SEQ ID NO: 2; 17-417 of SEQ ID NO: 2; 16-417 of SEQ ID NO: 2; 15-417 of SEQ ID NO: 2; 14-417 of SEQ ID NO: 2; 13-417 of SEQ ID NO: 2; 12-417 of SEQ ID NO: 2; 11-417 of SEQ ID NO: 2; 10-417 of SEQ ID NO: 2; 9-417 of SEQ ID NO: 2; 8-417 of SEQ ID NO: 2; 7-417 of SEQ ID NO: 2; 6-417 of SEQ ID NO: 2; 5-417 of SEQ ID NO: 2; 4-417 of SEQ ID NO: 2; 3-417 of SEQ ID NO: 2; 2-417 of SEQ ID NO: 2; 1-417 of SEQ ID NO: 2; and a combination of two or more of said amino acid sequences, wherein said Sema6A polypeptide binds to plexin-A2 polypeptides.

[0070] Further embodiments include human Sema6A polypeptides for use in the methods of the present invention include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein said reference amino acid sequence is selected from the group consisting of amino acids 40-472 of SEQ ID NO: 2; 41-472 of SEQ ID NO: 2; 42-472 of SEQ ID NO: 2; 43-472 of SEQ ID NO: 2; 44-472 of SEQ ID NO: 2; 45-472 of SEQ ID NO: 2; 46-472 of SEQ ID NO: 2; 47-472 of SEQ ID NO: 2; 48-472 of SEQ ID NO: 2; 49-472 of SEQ ID NO: 2; 50-472 of SEQ ID NO: 2; 51-472 of SEQ ID NO: 2; 52-472 of SEQ ID NO: 2; 53-472 of SEQ ID NO: 2; 54-472 of SEQ ID NO: 2; 55-472 of SEQ ID NO: 2; 56-472 of SEQ ID NO: 2; 57-472 of SEQ ID NO: 2; 58-472 of SEQ ID NO: 2; 59-472 of SEQ ID NO: 2; 60-472 of SEQ ID NO: 2; 56-465 of SEQ ID NO: 2; 56-466 of SEQ ID NO: 2; 56-467 of SEQ ID NO: 2; 56-468 of SEQ ID NO: 2; 56-469 of SEQ ID NO: 2; 56-470 of SEQ ID NO: 2; 56-471 of SEQ ID NO: 2; 56-472 of SEQ ID NO: 2; 56-473 of SEQ ID NO: 2; 56-474 of SEQ ID NO: 2; 56-475 of SEQ ID NO: 2; 56-476 of SEQ ID NO: 2; 56-477 of SEQ ID NO: 2;

56-478 of SEQ ID NO: 2; 56-479 of SEQ ID NO: 2; 56-480 of SEQ ID NO: 2; and a combination of two or more of said amino acid sequences. ,

[0071] Further embodiments include human Sema6A polypeptides for use in the methods of the present invention include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids 1-551 of SEQ ID NO: 2; 1-552 of SEQ ID NO: 2; 1-553 of SEQ ID NO: 2; 1-554 of SEQ ID NO: 2; 1-555 of SEQ ID NO: 2; 1-556 of SEQ ID NO: 2; 1-557 of SEQ ID NO: 2; 1-558 of SEQ ID NO: 2; 1-559 of SEQ ID NO: 2; 1-560 of SEQ ID NO: 2; 1-561 of SEQ ID NO: 2; 1-562 of SEQ ID NO: 2; 1-563 of SEQ ID NO: 2; 1-564 of SEQ ID NO: 2; 1-565 of SEQ ID NO: 2; 1-566 of SEQ ID NO: 2; 1-567 of SEQ ID NO: 2; 1-568 of SEQ ID NO: 2; 1-569 of SEQ ID NO: 2; 1-570 of SEQ ID NO: 2; 1-571 of SEQ ID NO: 2; 1-571 of SEQ ID NO: 2; 1-572 of SEQ ID NO: 2; 1-573 of SEQ ID NO: 2; 1-574 of SEQ ID NO: 2; 1-575 of SEQ ID NO: 2; 1-576 of SEQ ID NO: 2; 1-577 of SEQ ID NO: 2; 1-578 of SEQ ID NO: 2; 1-579 of SEQ ID NO: 2; 1-580 of SEQ ID NO: 2; 1-581 of SEQ ID NO: 2; 1-582 of SEQ ID NO: 2; 1-583 of SEQ ID NO: 2; 1-584 of SEQ ID NO: 2; 1-585 of SEQ ID NO: 2; 1-586 of SEQ ID NO: 2; 1-587 of SEQ ID NO: 2; 1-588 of SEQ ID NO: 2; 1-589 of SEQ ID NO: 2; 1-590 of SEQ ID NO: 2; 1-591 of SEQ ID NO: 2; 1-592 of SEQ ID NO: 2; 1-593 of SEQ ID NO: 2; 1-594 of SEQ ID NO: 2; 1-596 of SEQ ID NO: 2; 1-597 of SEQ ID NO: 2; 1-598 of SEQ ID NO: 2; 1-599 of SEQ ID NO: 2; 1-600 of SEQ ID NO: 2; and a combination of two or more of said amino acid sequences.

[0072] In other embodiments, human Sema6A polypeptides for use in the methods of the present invention include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids1-649 of SEQ ID NO: 2; 2-649 of SEQ ID NO: 2; 3-649 of -649 of SEQ ID NO: 2; 4-649 of SEQ ID NO: 2; 5-649 of SEQ ID NO: 2; 6-649 of SEQ ID NO: 2; 7-649 of SEQ ID NO: 2; 8-649 of SEQ ID NO: 2; 9-649 of SEQ ID NO: 2; 10-649 of SEQ ID NO: 2; 11-649 of SEQ ID NO: 2; 12-649 of SEQ ID NO: 2; 13-649 of SEQ ID NO: 2; 14-649 of SEQ ID NO: 2; 15-649 of SEQ ID NO: 2; 16-649 of SEQ ID NO: 2; 17-649 of SEQ ID NO: 2; 18-649 of SEQ ID NO: 2;

19-649 of SEQ ID NO: 2; 20-649 of SEQ ID NO: 2; 21-649 of SEQ ID NO: 2; 22-649 of SEQ ID NO: 2; 23-649 of SEQ ID NO: 2; 24-649 of SEQ ID NO: 2; 25-649 of SEQ ID NO: 2; 26-649 of SEQ ID NO: 2; and a combination of two or more of said amino acid sequences.

[0073] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids1-640 of SEQ ID NO: 2; 1-641 of SEQ ID NO: 2; 1-642 of SEQ ID NO: 2; 1-643 of SEQ ID NO: 2; 1-644 of SEQ ID NO: 2; 1-645 of SEQ ID NO: 2; 1-646 of SEQ ID NO: 2; 1-647 of SEQ ID NO: 2; 1-648 of SEQ ID NO: 2; 1-649 of SEQ ID NO: 2; 1-650 of SEQ ID NO: 2; 1-651 of SEQ ID NO: 2; 1-652 of SEQ ID NO: 2; 1-653 of SEQ ID NO: 2; 1-654 of SEQ ID NO: 2; 1-655 of SEQ ID NO: 2; 1-656 of SEQ ID NO: 2; 1-657 of SEQ ID NO: 2; 1-658 of SEQ ID NO: 2; 1-659 of SEQ ID NO: 2; 1-660 of SEQ ID NO: 2; 1-661 of SEQ ID NO: 2; 1-662 of SEQ ID NO: 2; 1-663 of SEQ ID NO: 2; 1-664 of SEQ ID NO: 2; 1-665 of SEQ ID NO: 2; 1-666 of SEQ ID NO: 2; 1-667 of SEQ ID NO: 2; 1-668 of SEQ ID NO: 2; 1-669 of SEQ ID NO: 2; 1-670 of SEQ ID NO: 2; and a combination of two or more of said amino acid sequences.

[0074] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids1-570 of SEQ ID NO: 4; 1-571 of SEQ ID NO: 4; 1-572 of SEQ ID NO: 4; 1-573 of SEQ ID NO: 4; 1-574 of SEQ ID NO: 4; 1-575 of SEQ ID NO: 4; 1-576 of SEQ ID NO: 4; 1-577 of SEQ ID NO: 4; 1-578 of SEQ ID NO: 4; 1-579 of SEQ ID NO: 4; 1-580 of SEQ ID NO: 4; 1-581 of SEQ ID NO: 4; 1-582 of SEQ ID NO: 4; 1-583 of SEQ ID NO: 4; 1-584 of SEQ ID NO: 4; 1-585 of SEQ ID NO: 4; 1-586 of SEQ ID NO: 4; 1-587 of SEQ ID NO: 4; 1-588 of SEQ ID NO: 4; 1-589 of SEQ ID NO: 4; 1-590 of SEQ ID NO: 4; and a combination of two or more of said amino acid sequences.

[0075] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid

sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids1-630 of SEQ ID NO: 4; 1-631 of SEQ ID NO: 4; 1-632 of SEQ ID NO: 4; 1-633 of SEQ ID NO: 4; 1-634 of SEQ ID NO: 4; 1-635 of SEQ ID NO: 4; 1-636 of SEQ ID NO: 4; 1-637 of SEQ ID NO: 4; 1-638 of SEQ ID NO: 4; 1-639 of SEQ ID NO: 4; 1-640 of SEQ ID NO: 4; 1-641 of SEQ ID NO: 4; 1-642 of SEQ ID NO: 4; 1-643 of SEQ ID NO: 4; 1-644 of SEQ ID NO: 4; 1-645 of SEQ ID NO: 4; 1-646 of SEQ ID NO: 4; 1-647 of SEQ ID NO: 4; 1-648 of SEQ ID NO: 4; 1-649 of SEQ ID NO: 4; 1-650 of SEQ ID NO: 4; 1-651 of SEQ ID NO: 4; 1-652 of SEQ ID NO: 4; 1-653 of SEQ ID NO: 4; 1-654 of SEQ ID NO: 4; 1-655 of SEQ ID NO: 4; 1-656 of SEQ ID NO: 4; 1-657 of SEQ ID NO: 4; 1-658 of SEQ ID NO: 4; 1-659 of SEQ ID NO: 4; 1-660 of SEQ ID NO: 4; 1-661 of SEQ ID NO: 4; 1-662 of SEQ ID NO: 4; 1-663 of SEQ ID NO: 4; 1-664 of SEQ ID NO: 4; 1-665 of SEQ ID NO: 4; 1-666 of SEQ ID NO: 4; and a combination of two or more of said amino acid sequences.

[0076] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids 45-492 of SEQ ID NO: 6; 46-492 of SEQ ID NO: 6; 47-492 of SEQ ID NO: 6; 48-492 of SEQ ID NO: 6; 49-492 of SEQ ID NO: 6; 50-492 of SEQ ID NO: 6; 51-492 of SEQ ID NO: 6; 52-492 of SEQ ID NO: 6; 53-492 of SEQ ID NO: 6; 54-492 of SEQ ID NO: 6; 55-492 of SEQ ID NO: 6; 56-492 of SEQ ID NO: 6; 57-492 of SEQ ID NO: 6; 58-492 of SEQ ID NO: 6; 59-492 of SEQ ID NO: 6; 60-492 of SEQ ID NO: 6; 61-492 of SEQ ID NO: 6; 56-485 of SEQ ID NO: 6; 56-486 of SEQ ID NO: 6; 56-487 of SEQ ID NO: 6; 56-488 of SEQ ID NO: 6; 56-489 of SEQ ID NO: 6; 56-490 of SEQ ID NO: 6; 56-491 of SEQ ID NO: 6; 56-492 of SEQ ID NO: 6; 56-493 of SEQ ID NO: 6; 56-494 of SEQ ID NO: 6; 56-495 of SEQ ID NO: 6; 56-496 of SEQ ID NO: 6; 56-497 of SEQ ID NO: 6; 56-498 of SEQ ID NO: 6; 56-599 of SEQ ID NO: 6; and a combination of two or more of said amino acid sequences.

[0077] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein the reference amino acid sequence is selected from the group

consisting of amino acids 1-580 of SEQ ID NO: 6; 1-581 of SEQ ID NO: 6; 1-583 of SEQ ID NO: 6; 1-584 of SEQ ID NO: 6; 1-585 of SEQ ID NO: 6; 1-586 of SEQ ID NO: 6; 1-587 of SEQ ID NO: 6; 1-588 of SEQ ID NO: 6; 1-589 of SEQ ID NO: 6; 1-590 of SEQ ID NO: 6; 1-591 of SEQ ID NO: 6; 1-592 of SEQ ID NO: 6; 1-593 of SEQ ID NO: 6; 1-594 of SEQ ID NO: 6; 1-595 of SEQ ID NO: 6; 1-596 of SEQ ID NO: 6; 1-597 of SEQ ID NO: 6; 1-598 of SEQ ID NO: 6; 1-599 of SEQ ID NO: 6; 1-600 of SEQ ID NO: 6; 2-590 of SEQ ID NO: 6; 3-590 of SEQ ID NO: 6; 4-590 of SEQ ID NO: 6; 5-590 of SEQ ID NO: 6; 6-590 of SEQ ID NO: 6; 7-590 of SEQ ID NO: 6; 8-590 of SEQ ID NO: 6; 9-590 of SEQ ID NO: 6; 10-590 of SEQ ID NO: 6; 11-590 of SEQ ID NO: 6; 12-590 of SEQ ID NO: 6; 13-590 of SEQ ID NO: 6; 14-590 of SEQ ID NO: 6; 15-590 of SEQ ID NO: 6; 16-590 of SEQ ID NO: 6; 17-590 of SEQ ID NO: 6; 18-590 of SEQ ID NO: 6; 19-590 of SEQ ID NO: 6; and a combination of two or more of said amino acid sequences.

[0078] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid sequence, wherein said reference amino acid sequence is selected from the group consisting of amino acids 56-580 of SEQ ID NO: 8; 56-581 of SEQ ID NO: 8; 56-583 of SEQ ID NO: 8; 56-584 of SEQ ID NO: 8; 56-585 of SEQ ID NO: 8; 56-586 of SEQ ID NO: 8; 56-587 of SEQ ID NO: 8; 56-588 of SEQ ID NO: 8; 56-589 of SEQ ID NO: 8; 56-590 of SEQ ID NO: 8; 56-591 of SEQ ID NO: 8; 56-592 of SEQ ID NO: 8; 56-593 of SEQ ID NO: 8; 56-594 of SEQ ID NO: 8; 56-595 of SEQ ID NO: 8; 56-596 of SEQ ID NO: 8; 56-597 of SEQ ID NO: 8; 56-598 of SEQ ID NO: 8; 56-599 of SEQ ID NO: 8; 56-600 of SEQ ID NO: 8; 56-601 of SEQ ID NO: 8; 56-602 of SEQ ID NO: 8; 56-603 of SEQ ID NO: 8; 56-604 of SEQ ID NO: 8; 56-605 of SEQ ID NO: 8; 45-595 of SEQ ID NO: 8; 46-595 of SEQ ID NO: 8; 47-595 of SEQ ID NO: 8; 48-595 of SEQ ID NO: 8; 49-595 of SEQ ID NO: 8; 50-595 of SEQ ID NO: 8; 51-595 of SEQ ID NO: 8; 52-595 of SEQ ID NO: 8; 53-595 of SEQ ID NO: 8; 54-595 of SEQ ID NO: 8; 55-595 of SEQ ID NO: 8; and a combination of two or more of said amino acid sequences.

[0079] The methods of the present invention further include a Sema6A polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence at least 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% identical to a reference amino acid

sequence, wherein the reference amino acid sequence is selected from the group consisting of amino acids 1-580 of SEQ ID NO: 8; 1-581 of SEQ ID NO: 8; 1-583 of SEQ ID NO: 8; 1-584 of SEQ ID NO: 8; 1-585 of SEQ ID NO: 8; 1-586 of SEQ ID NO: 8; 1-587 of SEQ ID NO: 8; 1-588 of SEQ ID NO: 8; 1-589 of SEQ ID NO: 8; 1-590 of SEQ ID NO: 8; 1-591 of SEQ ID NO: 8; 1-592 of SEQ ID NO: 8; 1-593 of SEQ ID NO: 8; 1-594 of SEQ ID NO: 8; 1-595 of SEQ ID NO: 8; 1-596 of SEQ ID NO: 8; 1-597 of SEQ ID NO: 8; 1-598 of SEQ ID NO: 8; 1-599 of SEQ ID NO: 8; 1-600 of SEQ ID NO: 8; 1-601 of SEQ ID NO: 8; 1-602 of SEQ ID NO: 8; 1-603 of SEQ ID NO: 8; 1-604 of SEQ ID NO: 8; 1-605 of SEQ ID NO: 8; 2-595 of SEQ ID NO: 8; 3-595 of SEQ ID NO: 8; 4-595 of SEQ ID NO: 8; 5-595 of SEQ ID NO: 8; 6-595 of SEQ ID NO: 8; 7-595 of SEQ ID NO: 8; 8-595 of SEQ ID NO: 8; 9-595 of SEQ ID NO: 8; 10-595 of SEQ ID NO: 8; 11-595 of SEQ ID NO: 8; 12-595 of SEQ ID NO: 8; 13-595 of SEQ ID NO: 8; 14-595 of SEQ ID NO: 8; 15-595 of SEQ ID NO: 8; 16-595 of SEQ ID NO: 8; 17-595 of SEQ ID NO: 8; 18-595 of SEQ ID NO: 8; 19-595 of SEQ ID NO: 8; and a combination of two or more of said amino acid sequences.

[0080] In certain embodiments, the Sema6A polypeptide of the present invention binds to plexin-A subfamily polypeptides. For example, the Sema6A polypeptide binds to a plexin-A1 polypeptide,a plexin-A2 polypeptide, a plexin-A3 polypeptide or a plexin-A4 polypeptide. In other embodiments, the Sema6A polypeptide may be isolated.

[0081] By "a reference amino acid sequence" is meant the specified sequence without the introduction of any amino acid substitutions. As one of ordinary skill in the art would understand, if there are no substitutions, the "isolated polypeptide" of the invention comprises an amino acid sequence which is identical to the reference amino acid sequence.

[0082] Sema6A polypeptides described herein may have various alterations such as substitutions, insertions or deletions. Exemplary amino acids that can be substituted in the polypeptide include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0083] Corresponding fragments of Sema6A polypeptides at least 70%, 75%, 80%, 85%, 90%, or 95% identical to the polypeptides and reference polypeptides described herein are also contemplated.

[0084] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0085] In methods of the present invention, a Sema6A polypeptide or polypeptide fragment of the invention may be administered directly as a preformed polypeptide. As discussed elsewhere herein, a Sema6A polypeptide may also be administered as a polynucleotide to be taken up by cells and expressed therein. For example, a polynucleotide encoding Sema6A may be administered as a viral vector.

Treatment Methods Using Sema6A polypeptides

[0086] One embodiment of the present invention provides methods for treating a disease, disorder or injury associated with dysmyelination or demyelination, *e.g.*, multiple sclerosis, in an animal suffering from such disease, the method comprising, consisting essentially of, or consisting of administering to the animal an effective amount of a Sema6A polypeptide or fragment thereof, a soluble Sema6A polypeptide, or variants, derivatives or analogs thereof.

[0087] Additionally, the invention is directed to a method for promoting myelination of neurons in a mammal comprising, consisting essentially of, or consisting of administering a therapeutically effective amount of a Sema6A polypeptide or a fragment thereof, a soluble Sema6A polypeptide, and variants, derivatives, or analogs thereof.

[0088] An additional embodiment of the present invention provides methods for treating a disease, disorder or injury associated with oligodendrocyte death or lack of differentiation, *e.g.*, multiple sclerosis, Pelizaeus Merzbacher disease or globoid cell leukodystrophy (Krabbe's disease), in an animal suffering from such disease, the method comprising, consisting essentially of, or consisting of administering to the animal an effective amount of a Sema6A polypeptide or a fragment thereof, a soluble Sema6A polypeptide, and variants, derivatives, or analogs thereof.

[0089] Another aspect of the invention includes a method for promoting proliferation, differentiation and survival of oligodendrocytes in a mammal comprising, consisting essentially of, or consisting of administering a therapeutically effective amount of a Sema6A polypeptide or a fragment thereof, a soluble Sema6A polypeptide, and variants, derivatives, or analogs thereof.

[0090] The present invention is directed to a method for promoting proliferation, differentiation, or survival of oligodendrocytes, comprising contacting the oligodendrocytes with an effective amount of a composition comprising a Sema6A polypeptide. The present invention is further directed to a method for promoting oligodendrocyte-mediated myelination of neurons, comprising contacting a mixture of neurons and oligodendrocytes with an effective amount of a composition comprising an isolated Sema6A polypeptide.

[0091] A Sema6A polypeptide to be used in treatment methods disclosed herein, can be prepared and used as a therapeutic agent that induce, promote, activate, or stimulate the ability of Sema6A to regulate myelination of neurons by oligodendrocytes. Additionally, the Sema6A polypeptide to be used in treatment methods disclosed herein can be prepared and used as a therapeutic agent that induces, promotes, activates, or stimulates the ability of Sema6A to regulate oligodendrocyte differentiation, proliferation and survival.

[0092] Further embodiments of the invention include a method of inducing oligodendrocyte proliferation or survival to treat a disease, disorder or injury involving

the destruction of oligodendrocytes or myelin comprising administering to a mammal, at or near the site of the disease, disorder or injury, in an amount sufficient to reduce inhibition of axonal extension and promote myelination.

[0093] In another embodiment, the invention is directed to a method for promoting proliferation, differentiation, or survival of oligodendrocytes in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising an isolated polynucleotide which encodes a Sema6A polypeptide disclosed herein or a method for promoting myelination of neurons in a mammal, comprising administering to a mammal thereof an effective amount of a composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide disclosed herein.

[0094] The invention also includes a method for treating a disease, disorder, or injury associated with destruction of myelin or dysmyelination or demyelination or a disease disorder, or injury associated with oligodendrocyte death or lack of differentiation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising an isolated polynucleotide which encodes a Sema6A polypeptide.

[0095] In methods of the present invention, a Sema6A polypeptide can be administered via direct administration of a Sema6A polypeptide to the patient. Alternatively, the Sema6A polypeptide can be administered via an expression vector which produces the specific Sema6A polypeptide. In certain embodiments of the invention, a Sema6A polypeptide is administered in a treatment method that includes: (1) transforming or transfecting an implantable host cell with a nucleic acid, *e.g.*, a vector, that expresses a Sema6A polypeptide; and (2) implanting the transformed host cell into a mammal, at the site of a disease, disorder or injury. For example, the transformed host cell can be implanted at the site of a chronic lesion of MS. In some embodiments of the invention, the implantable host cell is removed from a mammal, temporarily cultured, transformed or transfected with an isolated nucleic acid encoding a Sema6A polypeptide, and implanted back into the same mammal from which it was removed. The cell can be, but is not required to be, removed from the same site at which it is implanted. Such embodiments, sometimes known as *ex vivo* gene therapy, can provide a continuous supply of the Sema6A polypeptide, localized at the site of action, for a limited period of time.

[0096] Diseases or disorders which may be treated or ameliorated by the methods of the present invention include diseases, disorders or injuries which relate to dysmyelination or demyelination of mammalian neurons. Specifically, diseases and disorders in which the myelin which surrounds the neuron is either absent, incomplete, not formed properly or is deteriorating. Such diseases include, but are not limited to, multiple sclerosis (MS) including relapsing remitting, secondary progressive and primary progressive forms of MS; progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), adrenoleukodystrophy, Alexander's disease, Pelizaeus Merzbacher disease (PMZ), globoid cell leukodystrophy (Krabbe's disease), Wallerian Degeneration, optic neuritis and transvere myelitis.

[0097] Diseases or disorders which may be treated or ameliorated by the methods of the present invention include neurodegenerate disease or disorders. Such diseases include, but are not limited to, amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease and Parkinson's disease.

[0098] Examples of additional diseases, disorders or injuries which may be treated or ameliorated by the methods of the present invention include, but are not limited, to spinal cord injuries, chronic myelopathy or rediculopathy, tramatic brain injury, motor neuron disease, axonal shearing, contusions, paralysis, post radiation damage or other neurological complications of chemotherapy, stroke, large lacunes, medium to large vessel occlusions, leukoariaosis, acute ischemic optic neuropathy, vitamin E deficiency (isolated deficiency syndrome, AR, Bassen-Kornzweig syndrome), B12, B6 (pyridoxine - pellagra), thiamine, folate, nicotinic acid deficiency, Marchiafava-Bignami syndrome, Metachromatic Leukodystrophy, Trigeminal neuralgia, Bell's palsy, or any neural injury which would require axonal regeneration, remylination or oligodendrocyte survival or differentiation/proliferation.

Fusion Proteins and Conjugated Polypeptides

[0099] Some embodiments of the invention involve the use of a Sema6A polypeptide fused to a heterologous polypeptide moiety to form a fusion protein. Such fusion proteins can be used to accomplish various objectives, *e.g.*, increased serum half-life, improved bioavailability, *in vivo* targeting to a specific organ or tissue type, improved recombinant expression efficiency, improved host cell secretion, ease of purification, and higher

avidity. Depending on the objective(s) to be achieved, the heterologous moiety can be inert or biologically active. Also, it can be chosen to be stably fused to the Sema6A polypeptide moiety of the invention or to be cleavable, *in vitro* or *in vivo*. Heterologous moieties to accomplish these other objectives are known in the art.

[00100] As an alternative to expression of a fusion protein, a chosen heterologous moiety can be preformed and chemically conjugated to the Sema6A polypeptide moiety of the invention. In most cases, a chosen heterologous moiety will function similarly, whether fused or conjugated to the Sema6A polypeptide moiety. Therefore, in the following discussion of heterologous amino acid sequences, unless otherwise noted, it is to be understood that the heterologous sequence can be joined the Sema6A polypeptide moiety in the form of a fusion protein or as a chemical conjugate (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, Sema6A polypeptides may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. *See, e.g.*, PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[00101] Sema6A polypeptides for use in the treatment methods disclosed herein include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule such that covalent attachment does not prevent the Sema6A polypeptide from inhibiting the biological function of Sema6A. For example, but not by way of limitation, the Sema6A polypeptides of the present invention may be modified *e.g.*, by glycosylation, acetylation, pegylation, phosphylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[00102] Sema6A polypeptides for use in the treatment methods disclosed herein can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. Sema6A polypeptides may be modified by natural processes, such as posttranslational processing, or by chemical modification techniques which are well

known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the Sema6A polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini, or on moieties such as carbohydrates. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given Sema6A polypeptide. Also, a given Sema6A polypeptide may contain many types of modifications. Sema6A polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic Sema6A polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (*See*, for instance, *Proteins - Structure And Molecular Properties*, T. E. Creighton, W. H. Freeman and Company, New York 2nd Ed., (1993); *Posttranslational Covalent Modification Of Proteins*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol 182*:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci 663*:48-62 (1992)).

[00103] The present invention also provides for fusion proteins comprising, consisting essentially of, or consisting of a Sema6A polypeptide fusion. In certain embodiments, the Sema6A fusion polypeptide binds to plexin-A2 or plexin-A4. In certain embodiments of the invention, a Sema6A polypeptide, *e.g.*, a Sema6A polypeptide comprising the Sema domains and PSI domain or the entire extracellular domain (corresponding to amino acids 1 to 649 of SEQ ID NO: 2), is fused to a heterologous polypeptide moiety to form a Sema6A fusion polypeptide

[00104] Pharmacologically active polypeptides may exhibit rapid *in vivo* clearance, necessitating large doses to achieve therapeutically effective concentrations in the body. In addition, polypeptides smaller than about 60 kDa potentially undergo glomerular filtration, which sometimes leads to nephrotoxicity. Fusion or conjugation of polypeptide fragments can be employed to reduce or avoid the risk of such nephrotoxicity. Various heterologous amino acid sequences, *i.e.*, polypeptide moieties or "carriers," for increasing the *in vivo* stability, *i.e.*, serum half-life, of therapeutic polypeptides are known.

[00105] Due to its long half-life, wide *in vivo* distribution, and lack of enzymatic or immunological function, essentially full-length human serum albumin (HSA), or an HSA fragment, is commonly used as a heterologous moiety. Through application of methods and materials such as those taught in Yeh *et al., Proc. Natl. Acad. Sci. USA 89*:1904-08 (1992) and Syed *et al., Blood 89*:3243-52 (1997), HSA can be used to form a Sema6A fusion polypeptide that displays pharmacological activity by virtue of the Sema6A moiety while displaying significantly increased *in vivo* stability, *e.g.*, 10-fold to 100-fold higher. The C-terminus of the HSA can be fused to the N-terminus of the Sema6A moiety. Since HSA is a naturally secreted protein, the HSA signal sequence can be exploited to obtain secretion of the Sema6A fusion protein into the cell culture medium when the fusion protein is produced in a eukaryotic, *e.g.*, mammalian, expression system.

[00106] The signal sequence is a polynucleotide that encodes an amino acid sequence that initiates transport of a protein across the membrane of the endoplasmic reticulum. Signal sequences useful for constructing an immunofusion include antibody light chain signal sequences, *e.g.*, antibody 14.18 (Gillies *et al., J. Immunol. Meth. 125*:191-202 (1989)), and antibody heavy chain signal sequences, *e.g.*, the MOPC141 antibody heavy chain signal sequence (Sakano *et al., Nature 286*:5774 (1980)). Alternatively, other signal sequences can be used. *See, e.g.*, Watson, *Nucl. Acids Res. 12*:5145 (1984). The signal peptide is usually cleaved in the lumen of the endoplasmic reticulum by signal peptidases. This results in the secretion of an immunofusin protein containing the Fc region and the Sema6A moiety.

[00107] In some embodiments, the DNA sequence may encode a proteolytic cleavage site between the secretion cassette and the Sema6A polypeptide. Such a cleavage site may provide, *e.g.*, for the proteolytic cleavage of the encoded fusion protein, thus separating the Fc domain from the target protein. Useful proteolytic cleavage sites include amino

acid sequences recognized by proteolytic enzymes such as trypsin, plasmin, thrombin, factor Xa, or enterokinase K.

[00108] The secretion cassette can be incorporated into a replicable expression vector. Useful vectors include linear nucleic acids, plasmids, phagemids, cosmids and the like. An exemplary expression vector is pdC, in which the transcription of the immunofusin DNA is placed under the control of the enhancer and promoter of the human cytomegalovirus. *See, e.g.*, Lo *et al., Biochim. Biophys. Acta 1088*:712 (1991); and Lo *et al., Protein Engineering 11*:495-500 (1998). An appropriate host cell can be transformed or transfected with a DNA that encodes a Sema6A polypeptide and used for the expression and secretion of the Sema6A polypeptide. Host cells that are typically used include immortal hybridoma cells, myeloma cells, 293 cells, Chinese hamster ovary (CHO) cells, Hela cells, and COS cells.

[00109] In one embodiment, a Sema6A polypeptide is fused to a hinge and Fc region, *i.e.*, the C-terminal portion of an Ig heavy chain constant region. Potential advantages of a Sema6A-Fc fusion include solubility, *in vivo* stability, and multivalency, *e.g.*, dimerization. The Fc region used can be an IgA, IgD, or IgG Fc region (hinge- C_H2-C_H3). Alternatively, it can be an IgE or IgM Fc region (hinge- C_H2- C_H3-C_H4). An IgG Fc region is generally used, *e.g.*, an IgG_1 Fc region or IgG_4 Fc region. In one embodiment, a sequence beginning in the hinge region just upstream of the papain cleavage site which defines IgG Fc chemically (*i.e.*, residue 216, taking the first residue of heavy chain constant region to be 114 according to the Kabat system), or analogous sites of other immunoglobulins is used in the fusion. The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion, or binding characteristics of the molecule. Materials and methods for constructing and expressing DNA encoding Fc fusions are known in the art and can be applied to obtain Sema6A fusions without undue experimentation. Some embodiments of the invention employ a fusion protein such as those described in Capon *et al.*, U.S. Patent Nos. 5,428,130 and 5,565,335.

[0100] Fully intact, wild-type Fc regions display effector functions that may be unnecessary and undesired in an Fc fusion protein used in the methods of the present invention. Therefore, certain binding sites typically are deleted from the Fc region during the construction of the secretion cassette. For example, since coexpression with the light

chain is unnecessary, the binding site for the heavy chain binding protein, Bip (Hendershot *et al.*, *Immunol. Today* 8:111-14 (1987)), is deleted from the CH2 domain of the Fc region of IgE, such that this site does not interfere with the efficient secretion of the immunofusin. Transmembrane domain sequences, such as those present in IgM, also are generally deleted.

[0101] The IgG_1 Fc region is most often used. Alternatively, the Fc region of the other subclasses of immunoglobulin gamma (gamma-2, gamma-3 and gamma-4) can be used in the secretion cassette. The IgG_1 Fc region of immunoglobulin gamma-1 is generally used in the secretion cassette and includes at least part of the hinge region, the C_H2 region, and the C_H3 region. In some embodiments, the Fc region of immunoglobulin gamma-1 is a C_H2-deleted-Fc, which includes part of the hinge region and the C_H3 region, but not the C_H2 region. A C_H2-deleted-Fc has been described by Gillies *et al.*, *Hum. Antibod. Hybridomas* 1:47 (1990). In some embodiments, the Fc region of one of IgA, IgD, IgE, or IgM, is used.

[0102] Sema6A-Fc fusion proteins can be constructed in several different configurations. In one configuration the C-terminus of the Sema6A moiety is fused directly to the N-terminus of the Fc hinge moiety. In a slightly different configuration, a short polypeptide, *e.g.*, 2-10 amino acids, is incorporated into the fusion between the N-terminus of the Sema6A moiety and the C-terminus of the Fc moiety. Such a linker provides conformational flexibility, which may improve biological activity in some circumstances. If a sufficient portion of the hinge region is retained in the Fc moiety, the Sema6A-Fc fusion will dimerize, thus forming a divalent molecule. A homogeneous population of monomeric Fc fusions will yield monospecific, bivalent dimers. A mixture of two monomeric Fc fusions each having a different specificity will yield bispecific, bivalent dimers.

[0103] Any of a number of cross-linkers that contain a corresponding amino-reactive group and thiol-reactive group can be used to link Sema6A polypeptides to serum albumin or other heterologous polypeptides. Examples of suitable linkers include amine reactive cross-linkers that insert a thiol-reactive maleimide, *e.g.*, SMCC, AMAS, BMPS, MBS, EMCS, SMPB, SMPH, KMUS, and GMBS. Other suitable linkers insert a thiol-reactive haloacetate group, *e.g.*, SBAP, SIA, SIAB. Linkers that provide a protected or non-protected thiol for reaction with sulfhydryl groups to product a reducible linkage

include SPDP, SMPT, SATA, and SATP. Such reagents are commercially available (*e.g.*, Pierce Chemicals).

[0104] Conjugation does not have to involve the N-terminus of a Sema6A polypeptide or the thiol moiety on serum albumin. For example, Sema6A-albumin fusions can be obtained using genetic engineering techniques, wherein the Sema6A moiety is fused to the serum albumin gene at its N-terminus, C-terminus, or both.

[0105] Sema6A polypeptides can be fused to heterologous peptides to facilitate purification or identification of the Sema6A moiety. For example, a histidine tag can be fused to a Sema6A polypeptide to facilitate purification using commercially available chromatography media.

[0106] In some embodiments of the invention, a Sema6A fusion construct is used to enhance the production of a Sema6A moiety in bacteria. In such constructs a bacterial protein normally expressed and/or secreted at a high level is employed as the N-terminal fusion partner of a Sema6A polypeptide. *See, e.g.*, Smith *et al., Gene 67*:31 (1988); Hopp *et al., Biotechnology 6*:1204 (1988); La Vallie *et al., Biotechnology 11*:187 (1993).

[0107] By fusing a Sema6A moiety at the amino and carboxy termini of a suitable fusion partner, bivalent or tetravalent forms of a Sema6A polypeptide can be obtained. For example, a Sema6A moiety can be fused to the amino and carboxy termini of an Ig moiety to produce a bivalent monomeric polypeptide containing two Sema6A moieties. Upon dimerization of two of these monomers, by virtue of the Ig moiety, a tetravalent form of a Sema6A protein is obtained. Such multivalent forms can be used to achieve increased binding affinity for the target. Multivalent forms of Sema6A also can be obtained by placing Sema6A moieties in tandem to form concatamers, which can be employed alone or fused to a fusion partner such as Ig or HSA.

[0108] In certain embodiments, Sema6A polypeptides for use in the methods of the present invention further comprise a targeting moiety. Targeting moieties include a protein or a peptide which directs localization to a certain part of the body, for example, to the brain or compartments therein. In certain embodiments, Sema6A polypeptides for use in the methods of the present invention are attached or fused to a brain targeting moiety. The brain targeting moieties are attached covalently (*e.g.*, direct, translational fusion, or by chemical linkage either directly or through a spacer molecule, which can be optionally cleavable) or non-covalently attached (*e.g.*, through reversible interactions

such as avidin, biotin, protein A, IgG, etc.). In other embodiments, the Sema6A polypeptides for use in the methods of the present invention thereof are attached to one more brain targeting moieties. In additional embodiments, the brain targeting moiety is attached to a plurality of Sema6A polypeptides for use in the methods of the present invention.

[0109] A brain targeting moiety associated with a Sema6A polypeptide enhances brain delivery of such a Sema6A polypeptide. A number of polypeptides have been described which, when fused to a protein or therapeutic agent, delivers the protein or therapeutic agent through the blood brain barrier (BBB). Non-limiting examples include the single domain antibody FC5 (Abulrob *et al., J. Neurochem.* 95, 1201-1214 (2005)); mAB 83-14, a monoclonal antibody to the human insulin receptor (Pardridge *et al., Pharmacol. Res.* 12, 807-816 (1995)); the B2, B6 and B8 peptides binding to the human transferrin receptor (hTfR) (Xia *et al., J. Virol.* 74, 11359-11366 (2000)); the OX26 monoclonal antibody to the transferrin receptor (Pardridge *et al., J. Pharmacol. Exp. Ther.* 259, 66-70 (1991)); and SEQ ID NOs: 1-18 of U.S. Patent No. 6,306,365. The contents of the above references are incorporated herein by reference in their entirety.

[0110] Enhanced brain delivery of a Sema6A composition is determined by a number of means well established in the art. For example, administering to an animal a radioactively labelled Sema6A polypeptide linked to a brain targeting moiety; determining brain localization; and comparing localization with an equivalent radioactively labelled Sema6A polypeptide that is not associated with a brain targeting moiety. Other means of determining enhanced targeting are described in the above references.

Conjugated Polymers (other than polypeptides)

[0111] Some embodiments of the invention involve a Sema6A polypeptide wherein one or more polymers are conjugated (covalently linked) to the Sema6A polypeptide. Examples of polymers suitable for such conjugation include polypeptides (discussed above), sugar polymers and polyalkylene glycol chains. Typically, but not necessarily, a polymer is conjugated to the Sema6A polypeptide for the purpose of improving one or more of the following: solubility, stability, or bioavailability.

[0112] The class of polymer generally used for conjugation to a Sema6A polypeptide is a polyalkylene glycol. Polyethylene glycol (PEG) is most frequently used. PEG moieties, *e.g.*, 1, 2, 3, 4 or 5 PEG polymers, can be conjugated to each Sema6A polypeptide to increase serum half life, as compared to the Sema6A polypeptide alone. PEG moieties are non-antigenic and essentially biologically inert. PEG moieties used in the practice of the invention may be branched or unbranched.

[0113] The number of PEG moieties attached to the Sema6A polypeptide and the molecular weight of the individual PEG chains can vary. In general, the higher the molecular weight of the polymer, the fewer polymer chains attached to the polypeptide. Usually, the total polymer mass attached to the Sema6A polypeptide is from 20 kDa to 40 kDa. Thus, if one polymer chain is attached, the molecular weight of the chain is generally 20-40 kDa. If two chains are attached, the molecular weight of each chain is generally 10-20 kDa. If three chains are attached, the molecular weight is generally 7-14 kDa.

[0114] The polymer, *e.g.*, PEG, can be linked to the Sema6A polypeptide through any suitable, exposed reactive group on the polypeptide. The exposed reactive group(s) can be, *e.g.*, an N-terminal amino group or the epsilon amino group of an internal lysine residue, or both. An activated polymer can react and covalently link at any free amino group on the Sema6A polypeptide. Free carboxylic groups, suitably activated carbonyl groups, hydroxyl, guanidyl, imidazole, oxidized carbohydrate moieties and mercapto groups of the Sema6A polypeptide (if available) also can be used as reactive groups for polymer attachment.

[0115] In a conjugation reaction, from about 1.0 to about 10 moles of activated polymer per mole of polypeptide, depending on polypeptide concentration, is typically employed. Usually, the ratio chosen represents a balance between maximizing the reaction while minimizing side reactions (often non-specific) that can impair the desired pharmacological activity of the Sema6A polypeptide. Preferably, at least 50% of the biological activity (as demonstrated, *e.g.*, in any of the assays described herein or known in the art) of the Sema6A polypeptide is retained, and most preferably nearly 100% is retained.

[0116] The polymer can be conjugated to the Sema6A polypeptide using conventional chemistry. For example, a polyalkylene glycol moiety can be coupled to a lysine epsilon

amino group of the Sema6A polypeptide. Linkage to the lysine side chain can be performed with an N-hydroxylsuccinimide (NHS) active ester such as PEG succinimidyl succinate (SS-PEG) and succinimidyl propionate (SPA-PEG). Suitable polyalkylene glycol moieties include, *e.g.*, carboxymethyl-NHS and norleucine-NHS, SC. These reagents are commercially available. Additional amine-reactive PEG linkers can be substituted for the succinimidyl moiety. These include, *e.g.*, isothiocyanates, nitrophenylcarbonates (PNP), epoxides, benzotriazole carbonates, SC-PEG, tresylate, aldehyde, epoxide, carbonylimidazole and PNP carbonate. Conditions are usually optimized to maximize the selectivity and extent of reaction. Such optimization of reaction conditions is within ordinary skill in the art.

[0117] PEGylation can be carried out by any of the PEGylation reactions known in the art. *See, e.g., Focus on Growth Factors* 3:4-10 (1992), and European patent applications EP 0 154 316 and EP 0 401 384. PEGylation may be carried out using an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer).

[0118] PEGylation by acylation generally involves reacting an active ester derivative of polyethylene glycol. Any reactive PEG molecule can be employed in the PEGylation. PEG esterified to N-hydroxysuccinimide (NHS) is a frequently used activated PEG ester. As used herein, "acylation" includes without limitation the following types of linkages between the therapeutic protein and a water-soluble polymer such as PEG: amide, carbamate, urethane, and the like. *See, e.g., Bioconjugate Chem.* 5:133-140, 1994. Reaction parameters are generally selected to avoid temperature, solvent, and pH conditions that would damage or inactivate the Sema6A polypeptide.

[0119] Generally, the connecting linkage is an amide and typically at least 95% of the resulting product is mono-, di- or tri-PEGylated. However, some species with higher degrees of PEGylation may be formed in amounts depending on the specific reaction conditions used. Optionally, purified PEGylated species are separated from the mixture, particularly unreacted species, by conventional purification methods, including, *e.g.*, dialysis, salting-out, ultrafiltration, ion-exchange chromatography, gel filtration chromatography, hydrophobic exchange chromatography, and electrophoresis.

[0120] PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with Sema6A polypeptide in the presence of a reducing agent. In

addition, one can manipulate the reaction conditions to favor PEGylation substantially only at the N-terminal amino group of Sema6A polypeptide, *i.e.* a mono-PEGylated protein. In either case of mono-PEGylation or poly-PEGylation, the PEG groups are typically attached to the protein via a - C_H2-NH- group. With particular reference to the - C_H2- group, this type of linkage is known as an "alkyl" linkage.

[0121] Derivatization via reductive alkylation to produce an N-terminally targeted mono-PEGylated product exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization. The reaction is performed at a pH that allows one to take advantage of the pKa differences between the epsilon-amino groups of the lysine residues and that of the N-terminal amino group of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group, such as an aldehyde, to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs.

[0122] The polymer molecules used in both the acylation and alkylation approaches are selected from among water-soluble polymers. The polymer selected is typically modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled as provided for in the present methods. An exemplary reactive PEG aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10 alkoxy or aryloxy derivatives thereof (*see, e.g.*, Harris *et al.*, U.S. Pat. No. 5,252,714). The polymer may be branched or unbranched. For the acylation reactions, the polymer(s) selected typically have a single reactive ester group. For reductive alkylation, the polymer(s) selected typically have a single reactive aldehyde group. Generally, the water-soluble polymer will not be selected from naturally occurring glycosyl residues, because these are usually made more conveniently by mammalian recombinant expression systems.

[0123] Methods for preparing a PEGylated Sema6A polypeptide generally includes the steps of (a) reacting a Sema6A polypeptide with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the molecule becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions will be determined

case-by-case based on known parameters and the desired result. For example, a larger the ratio of PEG to protein, generally leads to a greater the percentage of poly-PEGylated product.

[0124] Reductive alkylation to produce a substantially homogeneous population of mono-polymer/Sema6A polypeptide generally includes the steps of: (a) reacting a Sema6A protein or polypeptide with a reactive PEG molecule under reductive alkylation conditions, at a pH suitable to pen-nit selective modification of the N-terminal amino group of the polypeptide; and (b) obtaining the reaction product(s).

[0125] For a substantially homogeneous population of mono-polymer/Sema6A polypeptide, the reductive alkylation reaction conditions are those that permit the selective attachment of the water-soluble polymer moiety to the N-terminus of the polypeptide. Such reaction conditions generally provide for pKa differences between the lysine side chain amino groups and the N-terminal amino group. For purposes of the present invention, the pH is generally in the range of 3-9, typically 3-6.

[0126] Sema6A polypeptides can include a tag, *e.g.*, a moiety that can be subsequently released by proteolysis. Thus, the lysine moiety can be selectively modified by first reacting a His-tag modified with a low-molecular-weight linker such as Traut's reagent (Pierce) which will react with both the lysine and N-terminus, and then releasing the His tag. The polypeptide will then contain a free SH group that can be selectively modified with a PEG containing a thiol-reactive head group such as a maleimide group, a vinylsulfone group, a haloacetate group, or a free or protected SH.

[0127] Traut's reagent can be replaced with any linker that will set up a specific site for PEG attachment. For example, Traut's reagent can be replaced with SPDP, SMPT, SATA, or SATP (Pierce). Similarly one could react the protein with an amine-reactive linker that inserts a maleimide (for example SMCC, AMAS, BMPS, MBS, EMCS, SMPB, SMPH, KMUS, or GMBS), a haloacetate group (SBAP, SIA, SIAB), or a vinylsulfone group and react the resulting product with a PEG that contains a free SH.

[0128] In some embodiments, the polyalkylene glycol moiety is coupled to a cysteine group of the Sema6A polypeptide. Coupling can be effected using, *e.g.*, a maleimide group, a vinylsulfone group, a haloacetate group, or a thiol group.

[0129] Optionally, the Sema6A polypeptide is conjugated to the polyethylene-glycol moiety through a labile bond. The labile bond can be cleaved in, *e.g.*, biochemical

hydrolysis, proteolysis, or sulfhydryl cleavage. For example, the bond can be cleaved under *in vivo* (physiological) conditions.

[0130] The reactions may take place by any suitable method used for reacting biologically active materials with inert polymers, generally at about pH 5-8, *e.g.*, pH 5, 6, 7, or 8, if the reactive groups are on the alpha amino group at the N-terminus. Generally the process involves preparing an activated polymer and thereafter reacting the protein with the activated polymer to produce the protein suitable for formulation.

Vectors

[0131] Vectors comprising nucleic acids encoding Sema6A polypeptides may also be used for the methods of the invention. The choice of vector and expression control sequences to which such nucleic acids are operably linked depends on the functional properties desired, *e.g.*, protein expression, and the host cell to be transformed.

[0132] Expression control elements useful for regulating the expression of an operably linked coding sequence are known in the art. Examples include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. When an inducible promoter is used, it can be controlled, *e.g.*, by a change in nutrient status, or a change in temperature, in the host cell medium.

[0133] The vector can include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally in a bacterial host cell. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Examples of bacterial drug-resistance genes are those that confer resistance to ampicillin or tetracycline.

[0134] Vectors that include a prokaryotic replicon can also include a prokaryotic or bacteriophage promoter for directing expression of the coding gene sequences in a bacterial host cell. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment to be expressed. Examples of such plasmid vectors are pUC8, pUC9, pBR322 and pBR329 (BioRad), pPL and pKK223 (Pharmacia). Any suitable prokaryotic host can

be used to express a recombinant DNA molecule encoding a protein used in the methods of the invention.

[0135] For the purposes of this invention, numerous expression vector systems may be employed. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. The neomycin phosphotransferase (neo) gene is an example of a selectable marker gene (Southern *et al.*, *J. Mol. Anal. Genet. 1*:327-341 (1982)). Additional elements may also be needed for optimal synthesis of mRNA. These elements may include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0136] In one embodiment, a proprietary expression vector of Biogen IDEC, Inc., referred to as NEOSPLA (U.S. patent 6,159,730) may be used. This vector contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression upon transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification. Of course, any expression vector which is capable of eliciting expression in eukaryotic cells may be used in the present invention. Examples of suitable vectors include, but are not limited to plasmids pcDNA3, pHCMV/Zeo, pCR3.1, pEF1/His, pIND/GS, pRc/HCMV2, pSV40/Zeo2, pTRACER-HCMV, pUB6/V5-His, pVAX1, and pZeoSV2 (available from Invitrogen, San Diego, CA), and plasmid pCI (available from Promega, Madison, WI). Additional eukaryotic cell expression vectors are known in the art and are commercially available. Typically, such vectors contain convenient restriction sites for insertion of the desired DNA segment. Exemplary vectors

include pSVL and pKSV-10 (Pharmacia), pBPV-1, pml2d (International Biotechnologies), pTDT1 (ATCC 31255), retroviral expression vector pMIG and pLL3.7, adenovirus shuttle vector pDC315, and AAV vectors. Other exemplary vector systems are disclosed *e.g.*, in U.S. Patent 6,413,777.

[0137] In general, screening large numbers of transformed cells for those which express suitably high levels of the polypeptide is routine experimentation which can be carried out, for example, by robotic systems.

[0138] Frequently used regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdmlP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, *see e.g.*, Stinski, U.S. Pat. No. 5,168,062; Bell, U.S. Pat. No. 4,510,245; and Schaffner, U.S. Pat. No. 4,968,615.

[0139] The recombinant expression vectors may carry sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (*see, e.g.*, Axel, U.S. Pat. Nos. 4,399,216; 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to a drug, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Frequently used selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[0140] Vectors encoding Sema6A polypeptides can be used for transformation of a suitable host cell. Transformation can be by any suitable method. Methods for introduction of exogenous DNA into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In

addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors.

[0141] Transformation of host cells can be accomplished by conventional methods suited to the vector and host cell employed. For transformation of prokaryotic host cells, electroporation and salt treatment methods can be employed (Cohen *et al., Proc. Natl. Acad. Sci. USA 69*:2110-14 (1972)). For transformation of vertebrate cells, electroporation, cationic lipid or salt treatment methods can be employed. *See, e.g.*, Graham *et al., Virology 52*:456-467 (1973); Wigler *et al., Proc. Natl. Acad. Sci. USA 76*:1373-76 (1979).

[0142] The host cell line used for protein expression can be of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to NSO, SP2 cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

[0143] Expression of polypeptides from production cell lines can be enhanced using known techniques. For example, the glutamine synthetase (GS) system is commonly used for enhancing expression under certain conditions. *See, e.g.*, European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

Host Cells

[0144] Host cells for expression of a Sema6A polypeptide for use in a method of the invention may be prokaryotic or eukaryotic. Exemplary eukaryotic host cells include, but are not limited to, yeast and mammalian cells, *e.g.*, Chinese hamster ovary (CHO) cells (ATCC Accession No. CCL61), NIH Swiss mouse embryo cells NIH-3T3 (ATCC

Accession No. CRL1658), and baby hamster kidney cells (BHK). Other useful eukaryotic host cells include insect cells and plant cells. Exemplary prokaryotic host cells are *E. coli* and *Streptomyces*.

Gene Therapy

[0145] A Sema6A polypeptide can be produced *in vivo* in a mammal, *e.g.*, a human patient, using a gene-therapy approach to treatment of a nervous-system disease, disorder or injury in which promoting survival, proliferation and/or differentiation of oligodendrocytes or promoting myelination of neurons would be therapeutically beneficial. This involves administration of a suitable Sema6A polypeptide-encoding nucleic acid operably linked to suitable expression control sequences. Generally, these sequences are incorporated into a viral vector. Suitable viral vectors for such gene therapy include an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector, an Epstein Barr viral vector, a papovaviral vector, a poxvirus vector, a vaccinia viral vector, an adeno-associated viral vector and a herpes simplex viral vector. The viral vector can be a replication-defective viral vector. Adenoviral vectors that have a deletion in its E1 gene or E3 gene are typically used. When an adenoviral vector is used, the vector usually does not have a selectable marker gene.

Pharmaceutical Compositions

[0146] The Sema6A polypeptides used in the methods of the invention may be formulated into pharmaceutical compositions for administration to mammals, including humans. The pharmaceutical compositions used in the methods of this invention comprise pharmaceutically acceptable carriers, including, *e.g.*, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0147] The compositions used in the methods of the present invention may be administered by any suitable method, *e.g.*, parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. As described previously, Sema6A polypeptides used in the methods of the invention act in the nervous system to promote survival, proliferation and differentiation of oligodendrocytes and myelination of neurons. Accordingly, in the methods of the invention, the Sema6A polypeptides are administered in such a way that they cross the blood-brain barrier. This crossing can result from the physico-chemical properties inherent in the Sema6A polypeptide molecule itself, from other components in a pharmaceutical formulation, or from the use of a mechanical device such as a needle, cannula or surgical instruments to breach the blood-brain barrier. Where the Sema6A polypeptide is a molecule that does not inherently cross the blood-brain barrier, *e.g.*, a fusion to a moiety that facilitates the crossing, suitable routes of administration are, *e.g.*, intrathecal or intracranial, *e.g.*, directly into a chronic lesion of MS. Where the Sema6A polypeptide is a molecule that inherently crosses the blood-brain barrier, the route of administration may be by one or more of the various routes described below.

[0148] Sterile injectable forms of the compositions used in the methods of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile, injectable preparation may also be a sterile, injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a suspension in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such

as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0149] Parenteral formulations may be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions may be administered at specific fixed or variable intervals, *e.g.*, once a day, or on an "as needed" basis.

[0150] Certain pharmaceutical compositions used in the methods of this invention may be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also may be administered by nasal aerosol or inhalation. Such compositions may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

[0151] The amount of a Sema6A polypeptide that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the type of polypeptide used and the particular mode of administration. The composition may be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0152] The methods of the invention use a "therapeutically effective amount" or a "prophylactically effective amount" of a Sema6A polypeptide. Such a therapeutically or prophylactically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual. A therapeutically or prophylactically effective amount is also one in which any toxic or detrimental effects are outweighed by the therapeutically beneficial effects.

[0153] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular Sema6A polypeptide used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated.

Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0154] In the methods of the invention the Sema6A polypeptides are generally administered directly to the nervous system, intracerebroventricularly, or intrathecally, *e.g.* into a chronic lesion of MS. Compositions for administration according to the methods of the invention can be formulated so that a dosage of 0.001 – 10 mg/kg body weight per day of the Sema6A polypeptide is administered. In some embodiments of the invention, the dosage is 0.01 – 1.0 mg/kg body weight per day. In some embodiments, the dosage is 0.001 – 0.5 mg/kg body weight per day. In certain embodiments, the dosage is 5 mg/kg - 100 mg/kg body weight per day. In further embodiments of the invention, the dosage is 50 mg/kg - 500 mg/kg body weight per day. The present invention also includes the dosage of 100 mg/kg - 1 g/kg body weigth per day. Non-limiting examples of the dosage used in the methods of the present invention is selected from the group consisting of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 19, 20, 25, 30, 35, 40, 50, 60 70, 80 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 mg/kg body weight per day. The dosage used in the present invention may be 1 g/kg - 5 g/kg body weight per day. Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include, but are not limited to, 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly.

[0155] In certain embodiments, a subject can be treated with a nucleic acid molecule encoding a Sema6A polypeptide. Doses for nucleic acids range from about 10 ng to 1 g, 100 ng to 100 mg, 1 μg to 10 mg, or 30-300 μg DNA per patient. Doses for infectious viral vectors vary from 10-100, or more, virions per dose.

[0156] Supplementary active compounds also can be incorporated into the compositions used in the methods of the invention. For example, a Sema6A polypeptide or a fusion protein may be coformulated with and/or coadministered with one or more additional therapeutic agents.

[0157] The invention encompasses any suitable delivery method for a Sema6A polypeptide to a selected target tissue, including bolus injection of an aqueous solution or implantation of a controlled-release system. Use of a controlled-release implant reduces the need for repeat injections.

[0158] The Sema6A polypeptides used in the methods of the invention may be directly infused into the brain. Various implants for direct brain infusion of compounds are known and are effective in the delivery of therapeutic compounds to human patients suffering from neurological disorders. These include chronic infusion into the brain using a pump, stereotactically implanted, temporary interstitial catheters, permanent intracranial catheter implants, and surgically implanted biodegradable implants. *See, e.g.*, Gill *et al.*, *supra*; Scharfen *et al.*, "High Activity Iodine-125 Interstitial Implant For Gliomas," *Int. J. Radiation Oncology Biol. Phys. 24*(4):583-591 (1992); Gaspar *et al.*, "Permanent 125I Implants for Recurrent Malignant Gliomas," *Int. J. Radiation Oncology Biol. Phys. 43*(5):977-982 (1999); chapter 66, pages 577-580, Bellezza *et al.*, "Stereotactic Interstitial Brachytherapy," in Gildenberg *et al.*, Textbook of Stereotactic and Functional Neurosurgery, McGraw-Hill (1998); and Brem *et al.*, "The Safety of Interstitial Chemotherapy with BCNU-Loaded Polymer Followed by Radiation Therapy in the Treatment of Newly Diagnosed Malignant Gliomas: Phase I Trial," *J. Neuro-Oncology* 26:111-23 (1995).

[0159] The compositions may also comprise a Sema6A polypeptide dispersed in a biocompatible carrier material that functions as a suitable delivery or support system for the compounds. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles such as suppositories or capsules. Implantable or microcapsular sustained release matrices include polylactides (U.S. Patent No. 3,773,319; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22:547-56 (1985)); poly(2-hydroxyethyl-methacrylate), ethylene vinyl acetate (Langer *et al., J. Biomed. Mater. Res. 15*:167-277 (1981); Langer, *Chem. Tech. 12*:98-105 (1982)) or poly-D-(-)-3hydroxybutyric acid (EP 133,988).

[0160] In some embodiments of the invention, a Sema6A polypeptide is administered to a patient by direct infusion into an appropriate region of the brain. *See, e.g.*, Gill *et al.*, "Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease," *Nature Med.* 9: 589-95 (2003). Alternative techniques are available and may be applied to administer a Sema6A polypeptide according to the invention. For example, stereotactic placement of a catheter or implant can be accomplished using the Riechert-Mundinger unit and the ZD (Zamorano-Dujovny) multipurpose localizing unit. A contrast-enhanced computerized tomography (CT) scan, injecting 120 ml of omnipaque, 350 mg iodine/ml, with 2 mm slice thickness can allow three-dimensional multiplanar treatment planning (STP, Fischer, Freiburg, Germany). This equipment permits planning on the basis of magnetic resonance imaging studies, merging the CT and MRI target information for clear target confirmation.

[0161] The Leksell stereotactic system (Downs Surgical, Inc., Decatur, GA) modified for use with a GE CT scanner (General Electric Company, Milwaukee, WI) as well as the Brown-Roberts-Wells (BRW) stereotactic system (Radionics, Burlington, MA) can be used for this purpose. Thus, on the morning of the implant, the annular base ring of the BRW stereotactic frame can be attached to the patient's skull. Serial CT sections can be obtained at 3 mm intervals though the (target tissue) region with a graphite rod localizer frame clamped to the base plate. A computerized treatment planning program can be run on a VAX 11/780 computer (Digital Equipment Corporation, Maynard, Mass.) using CT coordinates of the graphite rod images to map between CT space and BRW space.

[0162] The methods of treatment of demyelination or dysmyelination disorders as described herein are typically tested *in vitro*, and then *in vivo* in an acceptable animal model, for the desired therapeutic or prophylactic activity, prior to use in humans. Suitable animal models, including transgenic animals, are will known to those of ordinary skill in the art. For example, *in vitro* assays to demonstrate the differentiation and survival effect of the Sema6A polypeptides are described herein. The effect of the Sema6A polypeptides on myelination of axons or oligodendrocyte differentiation can be tested *in vitro* as described in the Examples. Finally, *in vivo* tests can be performed by creating transgenic mice which express the Sema6A polypeptide or by administering the Sema6A polypeptide to mice or rats in models.

Diagnosis or monitoring of Neurodegenerative Disease

[0163] Some embodiments of the present invention are directed to a method for diagnosing or monitoring a neurological disease or condition in a subject by (a) obtaining a specimen such as a tissue or a biological fluid sample, *e.g.*, blood or CSF, from the subject to be diagnosed or monitored, (b) measuring the level of Sema6A polypeptide in the specimen, and (c) comparing the level of Sema6A polypeptide to a reference specimen.

[0164] By the term "diagnose" is meant to identify an individual as having a particular disease or condition. By the term "monitor" is meant to check constantly and/or periodically on a given condition or phenomenon. In one embodiment, the method for monitoring a neurodegenerative disease includes obtaining biological fluid samples at several time points at intervals as part of the monitoring of the patient during the treatment for neurodegenerative disease. In another embodiment, the method for monitoring a neurodegenerative disease includes obtaining biological fluid samples at several time points at intervals as part of the monitoring of the patient during the treatment for MS.

[0165] In one embodiment, the disease or condition that is to be diagnosed or monitored multiple sclerosis (MS). In other embodiments, the diseasr or condition may be selected from the group consisting of progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), adrenoleukodystrophy, Alexander's disease, Pelizaeus Merzbacher disease (PMZ), Globoid cell Leucodystrophy (Krabbe's disease), Wallerian Degeneration, optic neuritis, transverse myelitis, amylotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, spinal cord injury, traumatic brain injury, post radiation injury, neurologic complications of chemotherapy, stroke, acute ischemic optic neuropathy, vitamin E deficiency, isolated vitamin E deficiency syndrome, AR, Bassen-Kornzweig syndrome, Marchiafava-Bignami syndrome, metachromatic leukodystrophy, trigeminal neuralgia, and Bell's palsy.

[0166] Biological fluid samples include, but are not limited to, blood, urine and cerebrospinal fluid (CSF). Methods by which biological fluid samples may be obtained include, but are not limited to, tissue biopsy, venipuncture, urine collection and spinal tap. In one embodiment, the biological fluid sample is CSF or blood.

[0167] Tissues include, but are not limited to, epithelium, muscle tissue, connective tissue, or nervous tissue. In one embodiment, the tissue is an epithelium, *e.g.*, a part of skin tissue. In another embodiment, dendritic cells collected from a tissue or a biological fluid such as CSF or blood are used to detect the Sema6A expression.

[0168] The biological fluid sample is obtained from a subject. In some embodiments, the subject is a vertebrate. Vertebrates include but are not limited to humans, mice, rats, sheep, goats, pigs, cattle, horses, reptiles, fishes, amphibians, and in eggs of birds, reptiles and fish. In one embodiment, the subject is a human. In another embodiment, the subject is a human that has or is suspected of having a neurological disease selected from the list consisting of MS, progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), adrenoleukodystrophy, Alexander's disease, Pelizaeus Merzbacher disease (PMZ), Globoid cell Leucodystrophy (Krabbe's disease), Wallerian Degeneration, optic neuritis, transverse myelitis, amylotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, spinal cord injury, traumatic brain injury, post radiation injury, neurologic complications of chemotherapy, stroke, acute ischemic optic neuropathy, vitamin E deficiency, isolated vitamin E deficiency syndrome, AR, Bassen-Kornzweig syndrome, Marchiafava-Bignami syndrome, metachromatic leukodystrophy, trigeminal neuralgia, and Bell's palsy. In one particular embodiment, the subject is an MS patient who has recently suffered at least one condition selected from the group consisting of numbness, weakness, visual impairment, loss of balance, dizziness, urinary bladder urgency, fatigue, and depression. As used herein, "recently" can be within 3, 5, 7, 10, 14 or 21 days.

[0169] Levels of Sema6A expression in the specimen can be indicative of a diseased state, *e.g.*, the severity of the disease or condition, the propensity of the subject to contract the disease, the prognosis for the subject, or the efficacy of therapies against the disease.

[0170] The present invention further provides for methods to detect the presence of the Sema6A polypeptide in a specimen obtained from a subject. Any method known in the art for detecting proteins or mRNA can be used. Such methods include, but are not limited to Coomassie Blue staining, immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination, and complement assays. [Basic and Clinical Immunology, 217-262, Sites and Terr, eds., Appleton & Lange, Norwalk, CT, (1991), which is incorporated by

reference]. The method to detect Sema6A mRNA is well known in the art. Tuan Rocky, Recombinant Protein Protocols: Detection and Isolation (Methods in Molecular Biology) (Mthods in Molecular Biology) (1st ed. Humana Press, PA 1997). Non-limiting examples of such methods are Northern blotting, nuclease protection assays, *in situ* hybridization, or an RT-PCR.

[0171] Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, *e.g.*, enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays, Western blot analysis and the like.

[0172] In diagnosing or monitoring a neurological disease in a subject, the level of Sema6A polypeptide in the specimen can be compared to the level of Sema6A polypeptide in a reference specimen. A suitable reference specimen can include, but is not limited to, a tissue or a biological fluid sample from a neurologically normal individual. In one embodiment, the reference specimen is from a subject not afflicted with a neurodegenerative disease. In another embodiment, the reference specimen is from a subject not afflicted with MS. In addition, a known protein produced by the subject, such as albumin if measuring from serum or total protein may act as an internal standard or control.

Diagnostic kits

[0173] Diagnostic kits are also contemplated by the present invention. These kits allow for the detection, diagnosis or monitoring of neurodegenerative diseases. The single-test approach adopted by these diagnostic kits will reduce the time required to diagnose a neurodegenerative disease in an individual and/or reduce the time required to detect differentially expressed proteins in a patient's biological fluid sample when he/she is being monitored for disease progression and/or effects of disease treatment.

[0174] One embodiment of the present invention is directed to diagnostic kits for the detection, diagnosis or monitoring of a neurodegenerative disease in a patient using an

antibody or antigen binding fragment that specifically binds to a Sema6A polypeptide described herein and a detectable label. In another embodiment, the invention is directed to a diagnostic kit for the detection, diagnosis or monitoring of MS in a patient using an antibody or antigen binding fragment that specifically binds to a Sema6A polypeptide and a detectable label.

[0175] In some embodiments, the antibody is labeled with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. In embodiments that are labeled with a detectable enzyme, the antibody is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, horseradish peroxidase with hydrogen peroxide and diaminobenzidine. An antibody also may be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

[0176] The kits contemplated by the invention are intended to detect, diagnose or monitor neurodegenerative diseases in vertebrates including but not limited to humans, mice, rats, sheep, goats, pigs, cattle, horses, reptiles, fishes, amphibians, and in eggs of birds, reptiles and fish.

[0177] The diagnostic kits of the present invention comprise some or all of the essential reagents required to perform a desired immunoassay according to the present invention. The diagnostic kit may be presented in a commercially packaged form as a combination of one or more containers holding the necessary reagents. Such a kit may comprise an antibody of the present invention, in combination with several conventional kit components. Conventional kit components will be readily apparent to those skilled in the art and are disclosed in numerous publications, including, for example, Harlow and Lane; Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1988) which is incorporated herein by reference in its entirety. Conventional kit components may include such items as, for example, microtiter plates, buffers to maintain the pH of the assay mixture (such as, but not limited to Tris, HEPES, phosphate, carbonate etc.), conjugated second antibodies, such as peroxidase conjugated anti-mouse IgG (or any anti-IgG to the animal from which the first antibody was derived),

stabilizers, biocides, inert proteins, *e.g.*, bovine serum albumin, or the like, and other standard reagents.

[0178] The diagnostic kits of the present invention also can include kits which are suitable for use in the home as well as the clinic, doctor's office or laboratory. Examples of home testing kits can be found for example in U.S. 5,602,040, which is incorporated by reference herein.

[0179] The term "detection" as used herein in the context of detecting the presence of protein in a patient is intended to include the determining of the amount of protein or the ability to express an amount of protein in a patient, the estimation of prognosis in terms of probable outcome of a disease and prospect for recovery, the monitoring of the protein levels over a period of time as a measure of status of the condition, and the monitoring of protein levels for determining a preferred therapeutic regimen for the patient, *e.g.* one with neurodegenerative disease.

Examples

Example 1

Sema6A is involved in oligodendrocyte biology

[0180] Oligodendrocytes mature through several developmental stages from oligodendrocyte progenitor cells (which express NG2), differentiating into pre-myelinating oligodendrocytes (which express O1 and O4) and finally into mature myelinating oligodendrocytes (which express O1, O4, myelin basic protein (MBP), and anti-proteolipid protein (PLP)). Thus, by monitoring the presence and absence of the NG2, O1, O4, MBP, and PLP markers it is possible to determine a given cell's developmental stage and to evaluate the role of Sema6A polypeptides in oligodendrocyte biology. Oligodendrocyte transcription factor-2 (Olig-2) is also known to be expressed in oligodendrocyte lineage and thus is used as a marker to detect oligodendrocytes. *See* Yokoo *et al. Amer. J. of Path.*164: 1717-1725 (2004) (For a general review of oligodendrocyte biology, see, *e.g.*, Baumann and Pham-Dinh, *Physiol. Rev.* 81: 871-927 (2001); Bras *et al.*, *Int. J. Dev. Biol.* 49: 209-220 (2005).

[0181] Monoclonal antibodies against O4 and MBP were from Chemicon. Monoclonal antibody against PLP) (clone AA3, 1:10) was a gift from Pr. C. Luberzki. Yamamura *et al.*, *J. Neurochem.* 57(5):1671-80 (1991). Monoclonal antibody against Astrocyte precursor cell (APC) was from VWR international (Fontenay Sous Bois, France). Antibody against CNPase was from Sigma. Antibody against NG2 (AB5320) was from Chemicon. Antibody against human Sema6A was from R&D Systems (Minneapolis, MN). Antibodies against Na^{2+} and paranodine were from Sigma. Anti-myc antibody (9E10, 1:100) was from Santa Cruz Biotechnology (SC-40).

Sema6A mRNA is expressed in oligodendrocytes

[0182] The expression of Sema6A mRNA was analyzed by *in situ* hybridization in fresh frozen brain (sagittal sections) or spinal cord (coronal sections) of P1 and P15 mice. Swiss mice (Janvier, Le Genest Saint Isle, France) were anesthetized by inhalation of isofluorane foren (Abbott) and decapitated. Brains and optic nerves were frozen immediately in isopentane (-50°C) and stored at -80°C before hybridization. Tissue sections were postfixed for 10 min in 4% PFA, washed in PBS, pH 7.4, treated with proteinase K (10 µg/ml; Invitrogen, Carlsbad, CA) for 3-5 min, postfixed for 5 min in 4% PFA, washed in PBS, acetylated, and washed in PBS 1% Triton X-100. Slides were incubated for 2 hr at room temperature in hybridization buffer (50% formamide, 5x SSC, 1x Denhardt's, 250 µg/ml yeast tRNA, and 500 µg/ml herring sperm, pH 7.4), and then tissue sections were hybridized overnight at 72°C with digoxigenin-labeled Sema6A riboprobes (0.5 ng/µl). After hybridization, sections were rinsed for 2 hr in 2x SSC at 72°C and blocked in 0.1 M Tris, pH 7.5, 0.15 M NaCl (B1) containing 10% normal goat serum (NGS) for 1 hr at room temperature. After blocking, slides were incubated overnight at room temperature with anti-digoxigenin antibody conjugated with the alkaline phosphatase (1:5000; Roche Diagnostics) in B1 containing 1% NGS. After additional washes, the alkaline phosphatase activity was detected using nitroblue tetrazolium chloride (NBT) (337.5 µg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (175 µg/ml) (Roche Diagnostics). Sections were mounted in Mowiol (Calbiochem/Merck, Carlstadt, Germany). As shown in Figure 3, Sema6A mRNA is widely expressed in the P15 mouse CNS white matter by oligodendrocytes during postnatal development.

Sema6A protein is expressed in oligodendrocytes

[0183] The expression of Sema6A protein in oligodendrocytes on P15 mouse brain sections (from 4% PFA fixed brain) was confirmed by double immunostaining of Sema6A and PLP, Sema6A and APC (a marker for oligodendrocyte), and Sema6A and CNPase (a marker for oligodendrocyte and Schwann Cell). The sections were blocked for 1 hr at room temperature (RT) in PBS containing 0.2% gelatin (Prolabo, Fontenay-sous-Bois, France) and 0.25% Triton X-100 (PBS-G-T), and then incubated overnight at RT with primary antibodies, *i.e.*, anti-mouse Sema6A antibody, anti-PLP antibody, anti-APC antibody, and anti-CNPase antibody. Cultures were then fixed with 4% PFA at room temperature for 10 min, rinsed, and then saturated and permeabilized in PBS buffer containing 10% NGS and 0.2% Triton-X100 for 30 min. Secondary antibodies, *i.e.*, CY3-conjugated antibody for Sema6A and FITC-conjugated antibody for PLP, APC, and CNPase were diluted in PBS containing 10% NGS and 0.1% Triton X-100 for 1h and, after washing, incubated for 1h at RT with the secondary antibodies. After rinsing, cultures are mounted in Mowiol (Calbiochem/Merck, Carlstadt, Germany).

[0184] The double immunostaining showed that all Sema6A expressing cells also expressed APC, CNPase, or PLP in the white matter (data not shown.) However, some cells expressing PLP, APC, and CNPase do not express Sema6A. Thus Sema6A protein seems to be expressed in a subset of cells of the oligodendrocyte lineage or in oligodendrocytes at a specific precise stage of maturation. Therefore, co-immunolabelling obtained from Sema6A combined with oligodendrocyte markers, PLP, APC, or CNPase, confirmed the expression of Sema6A by oligodendrocytes cells *in vivo*.

[0185] On the same P15 brain sections (cerebellum, cortex), immunostaining with oligodendroglial specific proteins, *e.g.*, PLP, was combined with Sema6A *in situ* hybridization. The standard *in situ* hybridization was performed as shown above except shortened proteinase K digestion (10 μg/ml) to 2 min. After *in situ* hybridization with a Sema6A riboprobe, sections were rinsed in PBS-T, blocked for 1 hr at RT in PBS-G-T and incubated overnight at RT with the anti-PLP antibody (clone AA3) and were then incubated in a biotinylated rabbit anti-rat antibody (1:200; Dako, Glostrup, Denmark) and an HRP-conjugated streptavidin (1:400; Amersham). The sections were developed with a diaminobenzidine reaction (brown precipitate). All cells expressing Sema6A transcript

appeared in purple and were also surrounded by a brown precipitate indicating PLP expression (data not shown).

The expression of Sema6A protein is developmentally regulated.

[0186] The expression of Sema6A and APC on forebrain coronal sections from P15, P30 and P45 mice was also analyzed by double immunostaining, using anti-mouse Sema6A and anti-APC antibodies as described above. Strong APC expression was observed at all the different ages but the maximal co-labelling of APC/Sema6A cells was observed at P15, where 65% of the oligodendrocytes (APC^+ cells) expressed Sema6A (data not shown). The proportion of APC-positive cells also expressing Sema6A in the white matter decreased to 14% at P30 and fell to 8% at P45 (data not shown). This showed that Sema6A expression is developmentally regulated, reaching a maximum at P15, during the peak of myelination.

Example 2

Sema6A expression at various stages of oligodendrocyte differentiation

[0187] The expression of Sema6A was also shown *in vitro* in purified oligodendrocyte cultures. The cortex hemispheres of P0 to P5 mice were dissected and transferred to a culture medium consisting of DMEM supplemented with 10% calf serum. The tissue was dissociated by sieving through a 70 µm mesh nylon sieve in the culture medium. The cell suspension was dispensed in 100 mm diameter plastic tissue culture dishes coated with polyornithine. Oligodendrocyte precursor cells were detached selectively by gentle syringing the culture medium on the cell layer. Dislodged cells were then submitted to two successive preplating over a 12 hr period in non-coated plastic culture dishes to allow adhesion of remaining astrocytes and microglia. The non- and loosely adherent cells (oligodendrocytes) were subcultured in 60 mm plastic culture dishes. The cultures were stained either with anti-mouse Sema6A antibodies and anti-NG2 (a marker for oligodendrocyte progenitor cells) or anti-O4 (a marker of the oligodendrocyte lineage expressed from the pre-oligodendrocyte stage) and anti-MBP antibodies (a marker for mature oligodendrocytes). CY3 conjugated antibody were used as a secondary antibody to visualize Sema6A expression and FITC-conjugated ones for NG2, O4 and MBP. After

24 hours *in vitro*, different types of cells express NG2: some cells with a very undifferentiated morphology were only FITC-labelled ($NG2^+$) and thus negative for Sema6A, others more differentiated ones (more processes) expressed NG2 and low level of Sema6A in the cell body but not in the cell processes (data not shown). After 48 hours in vitro O4-positive cells highly expressed Sema6A (data not shown). After 72 hours *in vitro*, MBP-positive cells highly expressed Sema6A (data not shown). This shows that Sema6A is more highly expressed in differentiated (O4 and MBP^+) oligodendrocytes (as observed *in vivo*).

Example 3

Sema6A-knockout mice exhibit a decrease in myelinated axons

[0188] In order to generate Sema6A- knockout mice, a cassette encoding CD4 transmembrane domain-ß-galactosidase-neomycin phosphotransferase (TM-ß-geo) and human placental alkaline phosphatase (PLAP), separated by an internal ribosome entry site (IRES), was inserted in the 17^{th} intron of Sema6A as described in Leighton *et al.*, *Nature*, 410: 174-179. The remaining N-terminal portion of the Sema6A protein up to amino acid 623 (and thus lacking the transmembrane and cytoplasmic domains) was fused to ß-galactosidase and trapped in the endoplasmic reticulum.

[0189] In order to analyze delay in myelination, the nodes of Ranvier in Sema6A-deficient mice have been studied. The nodes of Ranvier express several well identified proteins that have a characteristic expression and function at the node. Antibodies raised against the proteins involved in the formation of the node of Ranvier, such as paranodin, were used to detect the expression of the proteins. Nodes are well organized structures, closely interacting between the axon to be myelinated and the oligodendrocytes. Due to the characteristic expression of the proteins on the nodes, different regions can be visualized: Na^{2+} voltage gated channels to visualize the central region of the node and paranodin to visualize two domains surrounding the central region of the node, which is called paranodin/Na^{2+} channel cluster. The expression of Na^{2+} channels and paranodin were visualized by immunohistochemistry in P16 mice optic nerves using anti- Na^{2+} channels and paranodin antibodies (data not shown). The immunohistochemistry showed that a significant decrease in the number of paranodin/Na^{2+} channel clusters (-40.54%;

n=3) in Sema6A-deficient mice (data not shown). This result suggests that P16 Sema6A-deficient mice have less myelinated axons than the wild-type.

Sema6A-knockout mice exhibit reduced PLP expression in oligodendrocytes

[0190] To determine if the differentiation or proliferation of oligodendrocytes was normal in Sema6A-deficient mice, three major axonal tracts, the anterior commissure (AC), the corpus callosum (CC) and the optic nerves (ON), were labeled by non radioactive *in situ* hybridization, and the number of PLP expressing cells were quantified. Three ages were analyzed for AC and CC, P16, P30 and P45 (3 animals for each) and P16 for the ON. No significant changes in the expression of PLP were observed in the CC at any ages (data not shown). However, the number of PLP expressing cells were decreased at P16 in the AC of Sema-/- mice (-43%) compared to wild-type littermate (Figure 4). This reduction at P16 is explained by a major reduction of the number of PLP expressing cells (-60%), but also accompanied by a 30% reduction of the surface of the AC. The reduction is less pronounced in the AC at P30 (-20%) and goes back to normal in adults as shown in Figure 4. Likewise, the expression of PLP in the P16 optic nerve also showed a similar reduction (-26%) (data not shown). However, no significant change was observed in the number of oligodendrocyte transcription factor 2 (Olig2) expressing cells, which belongs to the oligodendrocyte lineage, in the AC (data not shown). These results suggest a possible role for Sema6A in either the differentiation of oligodendrocytes *in vivo* or their ability to migrate and colonize axonal tracts.

Sema6A-deficient oligodendrocytes exhibit delayed differentiation.

[0191] Oligodendrocytes were purified from Sema6A-/- newborn mice and analyzed for their ability to differentiate by the method described in Bernard *et al., J. Neurosc. Res.* 65: 439-445, 2001. Briefly, whole brain hemispheres of P0-P10 mouse or rat were dissected in phosphate buffered saline and transferred to culture medium composed of DMEM (Invitrogen 31966047) supplemented with penicillin (50 units/ml), streptomycin (50 µg/ml) (Invitrogen 15140), calf serum (10%) (Gibco 16030074), 5ng/ml PDGFBB (Sigma P3201) and 5ng/ml bFGF (Sigma F0291). The dissociation was performed by sieving the tissue through a 70 µm mesh nylon (BD Biosciences) sieve in the culture medium. The cell suspension was dispensed in 100 mm diameter plastic tissue culture

dishes coated with polyornithine (Sigma P3655). The cultures were incubated at 37 °C in a water saturated incubator equilibrated with 95% air-5% CO_2. Culture medium was changed 4 days after seeding and twice a week thereafter. After 8-10 days oligodendrocyte precursor cells were detached selectively by gentle syringing the culture medium on the cell layer. Dislodged cells were then submitted to two successive preplating over a 12 hr period in non coated plastic culture dishes to allow adhesion of remaining astrocytes and microglia. The non- and loosely adherent cells were subcultured in 60 mm plastic culture dishes coated with polyornithine in a chemically defined medium containing 0.5% fetal calf serum (FCS), 10 μM insulin, 100 μg/ml transferrin, 0.5 μg/ml albumin, 2 μM progesterone, 100 μM putrescine, 40 ng/ml triiodothyronine, 40 ng/ml L-thyroxine, 40 nM d-biotin and 100 nM hydrocortisone. In the absence of additional mitogen, these subcultures give rise to an almost homogeneous cell population containing more than 90% Gal-C positive cells after 10 days. *See* Besnard *et al.*, *Int. J. Dev. Neurosci.* 7(4): 401-409, 1989. To maintain these cells at the oligodendrocyte progenitor cell (OPC) stage and to prevent premature differentiation before processing, PDGFAA (10 ng/ml) (rats) or PDGFBB (10ng/ml) and bFGF (10 ng/ml for rats and 20ng/ml for mice) were added to the culture medium. After mitogen withdrawal, OPC differentiation occurs within 24-72 hrs. Bogler *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87(16): 6368-6372, 1990; Durand *et al. EMBO J.* 16(2): 306-317, 1997. Sema6A-Fc protein purchased from R&D system was also added to the chemically defined medium.

[0192] The maturation stage of oligodendrocyte can be measured by the morphological complexity of cultured oligodendrocytes, *e.g.*, measuring the fractal dimension (FD) of the cell. *Id.* Figure 5 shows the FD quantification of purified oligodendrocytes (visualized by phase contrast or labeled with anti-O4 antibody) from Sema6A+/- and Sema6A-/- mice, after 24 hr and 48 hr *in vitro*. This showed that while the majority of Sema6A+/- oligodendrocytes have a FD of 1.25 after 48 hr, most Sema6A-/- oligodendrocytes have an FD of only 1.05-1.15 (n=3) (Figure 5A). The FD of Sema6A+/- and Sema6A-/- oligodendrocytes after 24 hr *in vitro* is similar to the FD of Sema6A-/- oligodendrocyte after 48 hr. (data not shown). These results demonstrate that oligodendrocyte differentiation is delayed in Sema6A-/- mice.

[0193] After 72 hr in culture, oligodendrocytes were immunostained with anti-O4 antibodies (a marker for differentiating oligodendrocytes) and anti-MBP antibody (a

marker for differentiated oligodendrocytes) to measure the oligodendrocyte differentiation. As secondary antibodies, FITC-conjugated antibody for O4 and CY3-conjugated antibody for MBP were used. Under the microscope, randomly chosen fields were analyzed. After 72 hr, the number of O4+/MBP+ cells in Sema6A-/- were decreased 40.09% compared to that of wild-type (data not shown). The *in vitro* data supports the conclusion that oligodendrocyte differentiation is delayed in oligodendrocytes lacking Sema6A.

Example 4

Sema6A-Fc promotes myelination *in vitro*

[0194] The role of Sema6A in myelination was examined *in vitro* by treating co-cultures of dorsal root ganglion (DRG) neurons and oligodendrocytes with Sema6A-Fc and testing for myelination by immunohistochemistry and western blot. For these studies, it was necessary to first generate primary cultures of DRG neurons and oligodendrocytes.

[0195] Sprague Dawley rats E14-E17 embryonic dorsal root ganglia were plated on coverslips coated with poly-L-lysine (100 μg/ml). They were grown for 2 weeks in Neurobasal medium (Invitrogen 21103049) supplemented with B27 (Invitrogen 17504). To remove proliferating glial cells, the cultures were pulsed twice with fluorodeoxyuridine (20 μM) for 1 week. Oligodendrocytes were prepared as described in Example 3.

[0196] For coculture studies, oligodendrocytes were added to DRG neuron drop cultures in the presence or absence of 100-300 ng/ml Sema6A-Fc (R&D systems, 1146-S6-025). The culture medium (Neurobasal medium supplemented with B27 and 100 ng/ml NGF) was changed, and fresh Sema6A-Fc was added to the cells every 3 days. To identify changes in myelination, 3-week-old cultures were labeled with anti-MBP antibody and were subjected to SDS-PAGE followed by western blot analysis.

[0197] Figure 6 shows that addition of Sema6A-Fc protein increased myelination in a dose dependent manner, from the negative control to 0.1 μg/ml and from 0.1 μg/ml to 0.3 μg/ml. Western blot also showed that MBP expression is increased by addition of Sema6A-Fc, *i.e.*, 0.1 μg/ml to 0.3 μg/ml (data not shown). These data thus indicates that Sema6A polypeptides can promote or induce myelination.

Example 5

Sema6A-Fc involved in remyelination *in vivo*

[0198] The exposure to cuprizone (a copper chelator) was used as an experimental model in which important demyelination can be reproducibly induced in large areas of the mouse brain. *See* Matsushima *et al.*, *Brain Pathol.* 11 (1), 107–116, 2001. Eight week-old mice were fed with 0.2% cuprizone in their diet during 6 weeks, which causes mature oligodendrocytes to die by apoptosis. The cell death is closely followed by the recruitment of microglia and phagocytosis of myelin. At the termination of cuprizone treatment, or even after continued cuprizone exposure, oligodendrocyte precursor cells start to proliferate and invade demyelinated areas. If the cuprizone treatment is terminated, an almost complete remyelination takes place after a few weeks. Using the cuprizone model, Sema6A expression during demyelination and remyelination can be analyzed. After the cuprizone exposure and then termination, a significant increase of the number of Sema6A-expressing oligodendrocytes was observed in the corpus callosum of cuprizone-treated mice, starting 3 weeks after the administration of cuprizone, peaking at 4 weeks (+310%, n=3) and then returning to the basal level of expression at 6 weeks (Figure 7). These Sema6A expressing cells in the lesion were all positive for olig-2. To characterize this upregulation of Sema6A-expression, BrdU was injected to cuprizone-treated animals one week prior to their sacrifice. Some of the Sema6A-expressing oligodendrocytes were BrdU positive, suggesting that they were recruited from progenitors that differentiated during the induction of demyelination by cuprizone.

Example 6

Sema6A may play a role in experimental allergic encephalomyelitis (EAE)

[0199] To induce EAE, a 20amino-acid peptide based on the mouse Myelin Oligodendrocyte Glycoprotein (MOG) sequence 35-55 was used. The day of initiation of EAE is referred as day 1 of the experiment, and starting from day 7, clinical assessment of EAE was performed daily, and mice were scored for the disease according to the following criteria: no disease (0); decreased tail tone (1); hind-limb weakness or partial paralysis (2); complete hind-limb paralysis (3); front- and hind-limb paralysis (4); and

moribund state (5). These scores reflect the evolution of the demyelinating disease. After the EAE inducement by the 20 amino acids peptide, Sema6A-/- mice developed less behavior defects than the control animals did. Mean scores of Sema6A-/- mice reached only a maximum of 0.5 while the mean scores of the control animals reached 3.5 (data not shown). Only 25% Sema6A-/- mice developed any signs of illness (data not shown). These experiments suggest that Sema6A may play a role in EAE induction and perhaps more widely auto-immune pathologies such as multiple sclerosis.

[0200] In addition, plexin A4 -/- mice were tested for EAE induction. *See* Yamamoto *et al., Int. Immunol.* DOI : 10.1093/intimm/dxn006 (Jan. 2008). While the Sema6A -/- mice showed EAE resistance as shown above, the plexin A4 -/- mice showed increased sensitivity to the EAE induction. *See id.* Furthermore, an *in vitro* T cell proliferation assay was conducted in both Sema6A -/- and plexin A4 -/- mice. *See id.* While plexin A4 -/- mice showed significantly increased T cell proliferation, Sema6A -/- mice showed no difference in T cell proliferation. *See id.* This information suggests that the EAE resistance in Sema6A-/- mice may not be due to the abnormality of the immune response in the Sema6A -/- mice.

Example 7

Sema6A polypeptide is expressed in human Multiple Scherosis Lesion Tissue

[0201] To determine whether Sema6A expression differs between human MS lesion tissues and non-lesion tissues, Sema6A expression was measured by a standard *in situ* hybridization and immunostaining in human MS tissues (neocortex samples). The MS tissues were obtained from the Fédération de Neurologie at the Salpêtrière Hospital, 75013 Paris. A standard *in situ* hybridization was performed . Tissues were fixed by immersion in 4% paraformaldehyde and embedded in paraffin. For *in situ* hybridization, tissue sections were first dewaxed in xylene (3X5min) and then rehydrated by passing successively the slices through a decreasing gradient of ethanol (100%, 80%, 70%, 50%) and finally water and PBS. Tissue sections were postfixed for 10 min in 4% PFA, washed in PBS, pH 7.4, treated with proteinase K (50 µg/ml; Invitrogen, Carlsbad, CA) for 15 min at 37°C, postfixed for 5 min in 4% PFA, washed in PBS, acetylated, and dehydrated in successive bath of ethanol (50%, 70%, 80%, 100%). Slides were incubated for 2 hr at

68°C in hybridization buffer and processes as shown in Example 1. Slides were incubated for 2 hr at room temperature in hybridization buffer (50% formamide, 5x SSC, 1x Denhardt's, 250 μg/ml yeast tRNA, and 500 μg/ml herring sperm, pH 7.4), and then tissue sections were hybridized overnight at 72 °C with digoxigenin-labeled Sema6A riboprobes (0.5 ng/μl). After hybridization, sections were rinsed for 2 hr in 2x SSC at 72 °C and blocked in 0.1 M Tris, pH 7.5, 0.15 M NaCl (B1) containing 10% normal goat serum (NGS) for 1 hr at room temperature. After blocking, slides were incubated overnight at room temperature with anti-digoxigenin antibody conjugated with the alkaline phosphatase (1:5000; Roche Diagnostics) in B1 containing 1% NGS. After additional washes, the alkaline phosphatase activity was detected using nitroblue tetrazolium chloride (NBT) (337.5 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (175 μg/ml) (Roche Diagnostics). As shown in Figure 8A and 8B, Sema6A mRNA is widely expressed in the human MS lesion tissue, but is not expressed in the non-lesion tissue.

[0202] Immunostaining of the MS lesion tissue and non-lesion tissue was also conducted to show that the human MS lesion tissue highly expresses Sema6A. The tissue sections were blocked for 1 hr at room temperature (RT) in PBS containing 0.2% gelatin (Prolabo, Fontenay-sous-Bois, France) and 0.25% Triton X-100 (PBS-G-T), and then incubated overnight at RT with anti-human Sema6A antibody from R&D systems (Minneapolis, MN). Sections were then incubated for 1hr at room temperature with CY3-conjugated antibody, (Jackson Immunoresearch] diluted in PBS-G-T. After rinsing in PBS-G-T (3x10min), sections were mounted in Mowiol (Calbiochem/Merck, Carlstadt, Germany). As shown in Figure 8C, the MS lesion tissue shows high level of Sema6A expression, where non-lesion tissue shows little or no Sema6A expression (Figures 8A and 8B).

[0203] In order to determine a possible use of Sema6A expression as a biomarker, the Sema6A expression was also measured in a tissue of non MS patients, and Sema6A was not expressed in non-MS patients (data not shown). It is well known that blood cells, *e.g.*, dendritic cells, express Sema6A. *See* Gautier *et al. Immunopath. Infect. Dis.* 168(2): 453-465 (2006). The dendritic cells are also known to be present in cerebrospinal fluid (CSF). *See* Pashenkov *et al. Brain* 124(3): 480-492 (2001).

[0204] In view of the Sema6A expression on dendritic cells and differential Sema6A expression between MS lesion tissue and non lesion tissue, a specimen such as a tissue, *e.g.*, skin tissue, or a body fluid, *e.g.*, blood or CSF, is collected from a person to test for

an MS disease state. The specimen is tested for expression of Sema6A by, *e.g.*, ELISA. The presence in the fluid of a particular level may be indicative of possible MS, or the potential to develop MS. In additional embodiments, such an assay may be used to measure the effectiveness of a particular MS therapy. If the serum or CSF exhibits expression of Sema6A, the person from which the serum was drawn may be suspected of MS. Alternatively, dendritic cells from the collected specimen can be concentrated, and then the Sema6A in the specimen can be measured using, *e.g.*, ELISA. According to this method, Sema6A detection can be used as a marker of actual or potential MS disease.

Example 8

Sema6A polypeptide interacts with plexin-A2 polypeptide

[0205] To determine the interaction between the Sema6A polypeptides and plexin-A2 polypeptides, a recombinant Fc-dimerized AP-tagged Sema6A ectodomain (extracelllular domain) (AP-Sema6Aect-Fc) was added into mouse fibroblastic line cells (L cells) expressing full-length mouse plexin-A2 polypeptides as described in Suto *et al.*, *J. Neurosci.* 25: 3628-3637 (2005). To make the recombinant Sema6A ectodomain, the sequence corresponding to the ectodomain of mouse Sema6A (Sema6Aect; amino acids 18-648) was amplified and inserted into the Aptag-4 vector (a gift from Dr. J. Flanagan, Harvard Medical School, Boston, MA) (AP-Sema6Aect). *See* Flanagan *et al.*, *Methods Enzymol* 327: 17-35 (2000). To dimerize recombinant proteins, a fragment encoding AP-Sema6Aect was inserted into pEF-Fc (AP-Sema6Aect-Fc; AP-Sema6Bect-Fc; the pEF-Fc expression vector was a gift from Dr. S. Nagata, Osaka University). Human embryonic kidney 293T (HEK293T) cells were transfected with pEF-AP-Sema6Aect-Fc (the pCAGGS expression vector was a gift from Dr. J. Miyazaki, Osaka University) using Lipofectamine Plus (Invitrogen, Carlsbad, CA) and cultured in DMEM containing 10% fetal bovine serum (FBS) for 5-7 d in 5% CO_2 at 37°C. Culture supernatants were collected and filtered using 0.22 μm filters.

[0206] In order to generate plexin-A2 expressing L cells, the cDNA encoding the full-length mouse plexin-A2 protein was flanked with the signal sequence of mouse Sema3A, added the myc-tag (GGEQKLISEEDL: SEQ ID NO: 17) at the N-terminus, and then ligated into the expression vector pCAGGS. L cells were cotransfected with the plexin-

A2-expression vector and pST-neoB (Katoh, *et al.*, *Cell Struct. Funct.* 12: 575-580, 1987) according to the calcium phosphate method (Chen and Okayama, *Mol. Cell Biol.* 7: 2745-2752, 1987) and selected with GENETICIN (GIBCO). L cells were cultured with DH10 culture medium. Cell lines that stably expressed the plexin-A2 proteins were isolated by immunostaining with the anti-myc antibody 9E10. *See* Evan, *et al.*, *Mol. Cell Biol.* 5: 3610-3616, 1985.

[0207] In order to show the binding of Sema6A to Plexin-A2, L-cells that stably express the full-length mouse plexin-A2 proteins were incubated with 250 µl of HBSS with 0.5 mg/ml BSA, 0.1% NaN_3, and 20 mM HEPES, pH 7.0 (HBHA solution) containing 1% FBS and the AP-Sema6Aect-Fc recombinant proteins (the culture supernatant) for 1 h on ice as described in Flanagan and Leder, *Cell* 63: 185-194 (1990). After removal of the HBHA solution, cells were treated with 250 µl of 10 mM Tris-HCl, pH 8.0, supplemented with 0.1% Triton X-100 to dissolve the recombinant proteins bound to the cell surface. The cell lysates were subjected to colorimetric analysis to measure AP activity as described in Flanagan and Leder, *Cell* 63: 185-194 (1990) and Flanagan *et al.*, *Methods Enzymol* 327: 17-35 (2000).

[0208] It was shown that the Fc-dimerized recombinant AP-tagged ectodomain of Sema6A bound with a high affinity to plexin-A2 expressing L cells. The dissociation constant (Kd) value for the interaction of Sema6A with plexin-A2 was 3.21 nM (data not shown). The Kd value was comparable to the Kd value for the interaction of Sema6A with plexin-A4, *i.e.*, 3.56 nM) described in Suto *et al.*, *J. Neurosci.* 25: 3628-3637 (2005).

Example 9

A single mutation in plexin-A2 can ablate the binding of Sema6A

[0209] In order to determine the binding site of plexin-A2/plexin-A4 to Sema6A, C57BL6/J mutant mice, *i.e.*, NMF454, were examined. NMF454 mice (A gift of Dr S. Ackerman, Jackson Labs, Bar Harbor, USA) were identified in a recessive, genome-wide N-ethyl N-nitrosourea (ENU) mutagenesis screen of C57BL6/J mice. Histological analysis of NML454 mutant mice revealed a hypercellular molecular layer of the cerebellum which appeared strikingly similar to that of plexin-A2 and Sema6A null mice Renaud *et al. Nature Neuroscience*, in press (2008).

[0210] To determine whether the NMF454 mutation occurred in either the plexin-A2 or Sema6A gene, a gene mapping using microsatellite markers was conducted. F2 offspring ($\bar{n}$=11), which showed the NMF454 phenotype were generated by an intercross of F1 progenies from a mapping cross (C57BL6/J X BALB/cBy), and the affected F2 offspring were identified histologically. The F2 offspring were then genotyped with the polymorphic microsatellite markers, D1Mit155 and D18Mit178, which are closely linked to the plexin-A2 and Sema6A genes, respectively. No linkage was found with D18Mit178 (X^2=1.2; P>0.5). However, tight linkage was observed with D1Mit155 (X^2=33.0; P<0.0001). This result, combined with the phenotypic analysis, suggested that the NMF454 mutation resided in the plexin-A2 gene (Plxna2).

[0211] To determine the exact location of the mutation, western blot analysis of plexin-A2 expression in the cerebellum and neocortex was conducted. The western blot analysis revealed that a band around 250 kDa was present in NMF454 homozygous mutants mice (n=2), wild type and NMF454 heterozygous controls (n=2) but not in the regular plexin-A2 knockout line as shown in Figure 9A. This data suggests that the ENU mutation did not result in a null allele or a truncated plexin-A2 protein. To further localize the mutation, all exons of the plexin-A2 gene (Plxna2) were fully sequenced from genomic DNA of NMF454 homozygous mutants (n=3) and wild type controls (n=2). This revealed a single nucleotide substitution of the cytosine at position 1187 by an adenine resulting in the replacement of the alanine (396) by a glutamic acid residue. Furthermore, an alignment of vertebrate plexin-A sequences revealed that this alanine, localized in the semaphorin domain, is evolutionarily conserved in both Sema6A receptors, *i.e.*, plexin-A2 and plexin-A4 proteins (Figure 9B). However, the alignment showed that the alanine (396) was absent in plexin-A1 and plexin-A3, which are not known to bind Sema6A (Figure 9B). *See* Suto *et al. J. Neurosci.* 25: 3628 (2005); *See also* Suto *et al. Neuron* 53: 535 (2007).

[0212] To determine whether the alanine (396) mutation in NMF454 homozygous mutants perturbs the plexin-A2 binding to Sema6A, a targeted mutagenesis was conducted to introduce into the plexin-A2 cDNA the same point mutation of the cytosine 1187 (GCG to GAG) using QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). The primers used for the mutagenesis were as follows:

Forward Primer (SEQ ID NO: 18)

5'-GCAGTGCACCAAGGAGCCTGTCCCAATCG-3'

Reverse Primer (SEQ ID NO: 19)

5'-CGATTGGGACAGGCTCCTTGGTGCACTGC-3'

The mutated construct (plexin-A2A396E) was fully sequenced, confirming that only the cytosine 1187 was replaced by adenine.

[0213] This plexin-A2A396E cDNA was then expressed in COS7 cells, and its ability to bind Sema6A-AP was tested using the same method shown in Example 8. *See* Suto *et al. Neuron* 53: 5354 (2007). The results of the binding assay showed that Sema6A-AP bound very strongly to COS7 cells expressing wild-type plexin-A2 (Figure 9C). However, Sema6A-AP did not bind at all to cells expressing plexin-A2A396E (Figure 9D) although both wildtype and mutant proteins appeared to be expressed at similar levels.

WHAT IS CLAIMED IS:

1. A method for promoting proliferation, differentiation, or survival of oligodendrocytes, comprising contacting said oligodendrocytes with an effective amount of a composition comprising an isolated semaphorin 6A ("Sema6A") polypeptide

2. A method for promoting oligodendrocyte-mediated myelination of neurons, comprising contacting a mixture of neurons and oligodendrocytes with an effective amount of a composition comprising an isolated Sema6A polypeptide.

3. A method for promoting proliferation, differentiation, or survival of oligodendrocytes in a mammal, comprising administering to a mammal in need thereof an effective amount of a composition comprising an isolated Sema6A polypeptide.

4. A method for promoting myelination of neurons in a mammal, comprising administering to a mammal thereof an effective amount of a composition comprising an isolated Sema6A polypeptide.

5. A method for treating a disease, disorder, or injury associated with dysmyelination or demyelination in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising an isolated Sema6A polypeptide.

6. A method for treating a disease, disorder, or injury associated with oligodendrocyte death or lack of differentiation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising an isolated Sema6A polypeptide.

7. A method for treating a disease, disorder, or injury involving the destruction of myelin in a mammal comprising administering a therapeutically effective amount of a composition comprising an isolated Sema6A polypeptide.

8. A method for promoting proliferation, differentiation, or survival of oligodendrocytes in a mammal, comprising administering to a mammal in need thereof an effective amount of a

composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide.

9. A method for promoting myelination of neurons in a mammal, comprising administering to a mammal thereof an effective amount of a composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide.

10. A method for treating a disease, disorder, or injury associated with dysmyelination or demyelination in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide.

11. A method for treating a disease, disorder, or injury associated with oligodendrocyte death or lack of differentiation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide.

12. A method for treating a disease, disorder, or injury involving the destruction of myelin in a mammal comprising administering a therapeutically effective amount of a composition comprising an isolated polynucleotide, which encodes a Sema6A polypeptide.

13. The method of any one of claims 1-12, wherein said Sema6A polypeptide binds to a plexin-A2 polypeptide.

14. The method of any one of claims 1-13 wherein said Sema6A polypeptide comprises an amino acid sequence at least 80% identical to a reference amino acid sequence selected from the group consisting of:

i. 56 to 417 of SEQ ID NO: 2;

ii. a to 417 of SEQ ID NO:2;

iii. b to 417 of SEQ ID NO:2;

iv. 1 to 417 of SEQ ID NO:2;

v. 56 to c of SEQ ID NO: 2;

vi. a to c of SEQ ID NO: 2;

vii. b to c of SEQ ID NO: 2;

viii. 1 to c of SEQ ID NO: 2;

ix. 56 to c' of SEQ ID NO: 6;

x. a to c' of SEQ ID NO: 6;

xi. b to c' of SEQ ID NO: 6;

xii. 1 to c' of SEQ ID NO: 6;

xiii. 56 to d of SEQ ID NO: 2;

xiv. a to d of SEQ ID NO: 2;

xv. b to d of SEQ ID NO: 2;

xvi. 1 to d of SEQ ID NO: 2;

xvii. 56 to d' of SEQ ID NO: 6;

xviii. a to d' of SEQ ID NO: 6;

xix. b to d' of SEQ ID NO: 6;

xx. 1 to d' of SEQ ID NO: 6;

xxi. 56 to e of SEQ ID NO: 2;

xxii. a to e of SEQ ID NO: 2;

xxiii. b to e of SEQ ID NO: 2;

xxiv. 1 to e of SEQ ID NO: 2;

xxv. 56 to e' of SEQ ID NO: 6;

xxvi. a to e' of SEQ ID NO: 6;

xxvii. b to e' of SEQ ID NO: 6;

xxviii. 1 to e' of SEQ ID NO: 6;

xxix. 56 to e" of SEQ ID NO: 8;

xxx. a to e" of SEQ ID NO: 8;

xxxi. b to e" of SEQ ID NO: 8;

xxxii. 1 to e" of SEQ ID NO: 8;

xxxiii. 56 to e''' of SEQ ID NO: 4;

xxxiv. a to e''' of SEQ ID NO: 4;

xxxv. b to e''' of SEQ ID NO: 6;

xxxvi. 1 to e''' of SEQ ID NO: 8;

xxxvii. 56 to f of SEQ ID NO: 2;

xxxviii. a to f of SEQ ID NO: 2;

xxxix. b to f of SEQ ID NO: 2;

xl. 1 to f of SEQ ID NO: 2;

xli. 56 to f' of SEQ ID NO: 6;

xlii. a to f' of SEQ ID NO: 6;

xliii. b to f' of SEQ ID NO: 6;

xliv. 1 to f' of SEQ ID NO: 6;

xlv. 56 to f'' of SEQ ID NO: 8;

xlvi. a to f'' of SEQ ID NO: 8;

xlvii. b to f'' of SEQ ID NO: 8;

xlviii. 1 to f'' of SEQ ID NO: 8;

xlix. 56 to f''' of SEQ ID NO: 4;

l. a to f''' of SEQ ID NO: 4;

li. b to f''' of SEQ ID NO: 4;

lii. 1 to f''' of SEQ ID NO: 4;

liii. 56 to 1000 of SEQ ID NO: 2;

liv. a to 1000 of SEQ ID NO: 2;

lv. b to 1000 of SEQ ID NO: 2;

lvi. 1 to 1000 of SEQ ID NO: 2;

lvii. 56 to 1047 of SEQ ID NO: 4;

lviii. a to 1047 of SEQ ID NO: 4;

lix. b to 1047 of SEQ ID NO: 4;

lx. 1 to 1047 of SEQ ID NO: 4;

lxi. 56 to 971 of SEQ ID NO: 6;

lxii. a to 971 of SEQ ID NO: 6;

lxiii. b to 971 of SEQ ID NO: 6;

lxiv. 1 to 971 of SEQ ID NO: 6;

lxv. 56 to 975 of SEQ ID NO: 8;

lxvi. a to 975 of SEQ ID NO: 8;

lxvii. b to 975 of SEQ ID NO: 8;

lxviii. 1 to 975 of SEQ ID NO: 8;

lxix. 19 to 417 of SEQ ID NO: 2;

lxx. 19 to 472 of SEQ ID NO: 2;

lxxi. 19 to 551 of SEQ ID NO: 2;

lxxii. 19 to 492 of SEQ ID NO: 6;

lxxiii. 19 to 647 of SEQ ID NO:2;

lxxiv. 19 to 588 of SEQ ID NO: 6;

lxxv. 19 to 592 of SEQ ID NO: 8;

lxxvi. 19 to 664 of SEQ ID NO: 4;

lxxvii. 56 to 472 of SEQ ID NO: 2;

lxxviii. 56 to 551 of SEQ ID NO: 2;

lxxix. 56 to 492 of SEQ ID NO: 6;

lxxx. 56 to 647 of SEQ ID NO:2;

lxxxi. 56 to 588 of SEQ ID NO: 6;

lxxxii. 56 to 592 of SEQ ID NO: 8;

lxxxiii. 56 to 664 of SEQ ID NO: 4;

lxxxiv. 1 to 649 of SEQ ID NO: 2;

lxxxv. 1 to 590 of SEQ ID NO: 6;

lxxxvi. 1 to 594 of SEQ ID NO: 8;

lxxxvii. 1 to 666 of SEQ ID NO: 4;

lxxxviii. 18 to 703 of SEQ ID NO: 2;

lxxxix. 18 to 644 of SEQ ID NO: 6;

xc. 18 to 648 of SEQ ID NO: 8;

xci. 18 to 720 of SEQ ID NO: 4;

xcii. 1 to 648 of SEQ ID NO: 2

xciii. 1 to 589 of SEQ ID NO: 6;

xciv. 1 to 593 of SEQ ID NO: 8;

xcv. 1 to 665 of SEQ ID NO: 4; and

xcvi. a combination of two or more of said amino acid sequences;

wherein a is any integer between 24 and 56, b is any integer between 19 and 21, c is any integer between 472 and 512, c' is any integer between 418 and 453, d is any integer between 514 and 569, d' is any integer between 455 and 510, e is any integer between 570 and 650, e' is any integer between 511 and 591, e" is any integer between 570 and 595, and e"' is any integer between 570 and 667; f is any integer between 647 and 671, f' is any integer between 588 and 612, f" is any integer between 592-616, and f"' is any integer between 664 and 688.

15. The method of claim 14, wherein said amino acid sequence is at least 90% identical to said reference amino acid sequence.

16. The method of claim 14 or 15, wherein said amino acid sequence is identical to said reference amino acid sequence.

17. The method of any one of claims 1-16, wherein said Sema6A polypeptide is a cyclic peptide.

18. The method of claim 17, wherein said cyclic peptide comprises a biotin molecule attached to the N-terminus and a cysteine residue attached to the C-terminus of said cyclic peptide.

19. The method of claim 17, wherein said cyclic peptide comprises a cysteine residue attached to the N- and C-terminus of said cyclic peptide, wherein said N-terminal cysteine residue is acetylated.

20. The method of claim 18 or 19, wherein said C-terminal cysteine has an NH_2 moiety attached.

21. The method of any one of claims 1-0, wherein said polypeptide is attached to a non-Sema6A moiety.

22. The method of claim 21, wherein said non-Sema6A moiety is a heterologous polypeptide fused to said Sema6A polypeptide.

23. The method of claim 22, wherein said heterologous polypeptide is selected from the group consisting of an immunoglobulin polypeptide or fragment thereof, a serum albumin polypeptide or fragment thereof, a targeting polypeptide, a reporter polypeptide, and a purification-facilitating polypeptide and a combination of two or more of said heterologous polypeptides.

24. The method of claim 23, wherein said heterologous polypeptide is selected from the group consisting of c-myc, human placental alkaline phosphatase, an immunoglobulin hinge and Fc region and a combination of two or more of the heterologous polypeptides.

25. The method of claim 21, wherein said non-Sema6A moiety is a polymer conjugated to Sema6A polypeptide.

26. The method of claim 25, wherein the polymer is selected from the group consisting of a polyalkylene glycol, a sugar polymer, and a polypeptide.

27. The method of claim 26, wherein the polymer is a polyalkylene glycol.

28. The method of claim 27, wherein the polyalkylene glycol is polyethylene glycol (PEG).

29. The method of claim 25, wherein said Sema6A polypeptide is conjugated to 1, 2, 3 or 4 polymers.

30. The method of any one of claims 25-29, wherein the total molecular weight of the polymers is from 5,000 Da to 100,000 Da.

31. The method of any one of claims 3-13, wherein said mammal has been diagnosed with a disease, disorder, or injury involving demyelination, dysmyelination, or neurodegeneration.

32. The method of claim 31, wherein said disease, disorder, or injury is selected from the group consisting of multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), encephalomyelitis (EPL), central pontine myelolysis (CPM), adrenoleukodystrophy, Alexander's disease, Pelizaeus Merzbacher disease (PMZ), Wallerian Degeneration, optic neuritis, transverse myelitis, amylotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, spinal cord injury, traumatic brain injury, post radiation injury, neurologic complications of chemotherapy, stroke, acute ischemic optic neuropathy, vitamin E deficiency, isolated vitamin E deficiency syndrome, AR, Bassen-Kornzweig syndrome, Marchiafava-Bignami syndrome, metachromatic leukodystrophy, trigeminal neuralgia, and Bell's palsy.

33. The method of claim 31, wherein said disease, disorder, or injury is multiple sclerosis (MS).

34. The method any one of claims 3-33, wherein said composition is administered by bolus injection or chronic infusion.

35. The method of claim 34, wherein said composition is administered directly into the central nervous system.

36. The method of claim 35, wherein said composition is administered directly into a chronic lesion of MS.

37. The method of any one of claims 1 or 2, when said contacting comprises (a) transfecting said oligodendrocytes with a polynucleotide which encodes said Sema6A polypeptide

through operable linkage to an expression control sequence, and (b) allowing expression of said Sema6A polypeptide.

38. The method of any one of claims 8-13, wherein said polynucleotide encodes said Sema6A polypeptide through operable linkage to an expression control sequence.

39. The method of claim 38, wherein said polynucleotide is administered as an expression vector.

40. The method of claim 39, wherein said expression vector is a viral vector.

41. The method of any one of claims 8-13, wherein said administering comprises (a) providing a cultured host cell comprising said polynucleotide, wherein said cultured host cell expresses said Sema6A polypeptide; and (b) introducing said cultured host cell into said mammal such that said Sema6A polypeptide is expressed in said mammal.

42. The method of claim 41, wherein said cultured host cell is introduced into said mammal at or near the site of the nervous-system disease, disorder or injury.

43. The method of claim 41 or 42, wherein said cultured host cell is made by a method comprising (a) transforming or transfecting a recipient host cell with the polynucleotide of claim 37 or 38 or the vector of claim 39 or 40, and (b) culturing said transformed or transfected host cell.

44. The method of any one of claims 41-43, wherein said cultured host cell is derived from the mammal to be treated.

45. The method of any one of claims 3-44, wherein said Sema6A polypeptide is expressed in an amount sufficient to reduce inhibition of oligodendrocyte proliferation, differentiation or survival at or near the site of the nervous system disease, disorder, or injury.

46. The method of any one of claims 3-45, wherein said Sema6A polypeptide is expressed in an amount sufficient to reduce inhibition of neuron myelination at or near the site of the nervous system disease, disorder, or injury.

47. The method of claim 40, wherein the viral vector is selected from the group consisting of an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentivirus vector, a baculovirus vector, a herpesvirus vector, a papovavirus vector, and a poxvirus vector.

48. The method of claim 47, wherein said herpesvirus vector is selected from the group consisting of a herpes simplex virus vector and an Epstein Barr virus vector.

49. The method of claim 47, wherein said poxvirus vector is a vaccinia virus vector.

50. The method of any one of claims 39, 40, or 47-49, wherein said vector is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and subcutaneous administration.