USE OF SEMAPHORIN-4D BINDING MOLECULES FOR MODULATION OF BLOOD BRAIN BARRIER PERMEABILITY

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing in ASCII text file (Name: "1843_068PC03_SequenceListing_ascii.txt"; Size: 33,807 bytes; and Date of Creation: October 10, 2012) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Semaphorin 4D (SEMA4D), also known as CD100, is a transmembrane protein (*e.g.*, SEQ ID NO: 1 (human); SEQ ID NO: 2 (murine)) that belongs to the semaphorin gene family. SEMA4D is expressed on the cell surface as a homodimer, but upon cell activation SEMA4D can be released from the cell surface via proteolytic cleavage to generate sSEMA4D, a soluble form of the protein, which is also biologically active. See Suzuki *et al.*, *Nature Rev. Immunol. 3*:159-167 (2003); Kikutani *et al.*, *Nature Immunol. 9*:17-23 (2008).

[0003] SEMA4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, SEMA4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). Its expression, however, is upregulated in these cells following activation by various immunological stimuli. The release of soluble SEMA4D from immune cells is also increased by cell activation.

[0004] SEMA4D has been implicated in the development of neurodegenerative diseases, autoimmune diseases, demyelinating diseases, and certain cancers. While the role of SEMA4D signaling through its receptors, *e.g.*, Plexin-B1, on angiogenesis is well-recognized, the effect of SEMA4D signaling on Blood Brain Barrier (BBB) remains unclear. This is important because changes in the permeability of the BBB have a profound influence on brain tissue and function. There remains, therefore, a need for

treatments for neuroinflammatory disorders that arise as a result of breakdown in the BBB, and, in particular, therapeutics that inhibit, suppress, prevent, reverse, or slow the breakdown of the BBB.

BRIEF SUMMARY OF THE INVENTION

[0005] Methods for using semaphorin-4d binding molecules for modulation of blood brain barrier permeability are disclosed herein. Evidence is presented demonstrating that SEAM4D can compromise the integrity of the BBB thereby increasing its permeability. According to aspects of the invention illustrated herein, there is provided a method for decreasing blood brain barrier permeability in a subject having a neuroinflammatory disorder including administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D), thereby decreasing blood brain barrier permeability in the subject.

[0006] According to aspects illustrated herein, there is provided a method of maintaining or increasing Claudin-5 expression in a subject having a neuroinflammatory disorder comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D), wherein the binding molecule maintains or increases Claudin-5 expression in the subject.

[0007] According to aspects illustrated herein, there is provided a method of decreasing blood brain barrier permeability in a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically inhibits semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor thereby decreasing blood brain barrier permeability in the subject.

[0008] According to aspects illustrated herein, there is provided a method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically inhibits semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor, wherein the binding molecule decreases permeability of the blood-brain barrier, thereby treating the subject.

[0009] According to aspects illustrated herein, there is provided a method of decreasing blood-brain barrier permeability in a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule competitively inhibits a

reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D.

[0010] According to aspects illustrated herein, there is provided a method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) and an isolated binding molecule which specifically binds to Plexin-B1, wherein the SEMA4D and Plexin-B1 binding molecules decrease permeability of the blood-brain barrier, thereby treating the subject.

[0011] According to aspects illustrated herein, there is provided a method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an inhibitor of semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor, wherein the inhibitor decreases permeability of the blood-brain barrier, thereby treating the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0012] FIGURE 1: Schematic of the dynamic *in vitro* BBB ("DIV-BBB") experimental protocol described in the Examples.

[0013] FIGURES 2: *In vitro* DIV-BBB model showing measurements of BBB integrity as reflected in transendothelial electrical resistance (TEER) in the presence of recombinant SEMA4D (0.05, 0.5, 5 or 50 μg/mL) and VX15/2503 Antibody ("VX15").

[0014] FIGURE 3: *In vitro* DIV-BBB model showing measurements of BBB integrity as reflected in transendothelial electrical resistance (TEER) during the formation of the BBB, the breakdown of BBB in the presence of recombinant SEMA4D (0.5, 5 or 50 μg/mL), and the restoration of the BBB in presence of VX15/2503 Antibody ("VX15"), but not isotype control ("Iso").

[0015] FIGURE 4: *In vitro* DIV-BBB model showing measurements of BBB integrity as reflected in transendothelial electrical resistance (TEER) during the formation of the BBB, the breakdown of BBB in the presence of 0.25, 2.5, or 25 μg/mL of control C35 antigen ("CTRL") or 50 μg/mL of recombinant SEMA4D, and the restoration of the BBB in presence of VX15/2503 Antibody ("VX15").

[0016] FIGURE 5: *In vitro* DIV-BBB model showing measurements of BBB integrity as reflected in transendothelial electrical resistance (TEER) during the formation of the

BBB, the breakdown of BBB in the presence of recombinant SEMA4D (50 μg/mL), and the restoration of the BBB in the presence of VX15/2503 Antibody ("VX15"), anti-Plexin-B1 antibody ("Anti-PLXNB1"), but not isotype control ("Iso").

[0017] FIGURE 6: *In vitro* DIV-BBB model showing measurements of BBB integrity as reflected in transendothelial electrical resistance (TEER) during the formation of the BBB, the breakdown of BBB in the presence of activated PBMC (10^6/ml) and flow cessation, and the restoration of the BBB in the presence of VX15/2503 Antibody or Isotype Control IgG.

[0018] FIGURE 7A-C: Results from the *in vivo* EAE model showing integrity of the BBB or loss thereof as reflected by immunostaining of fibrinogen ("Fib.+") penetration into brain tissue (7A left panel and quantitation in 7B) and Claudin-5 ("CLN5+") expression as detected by red stain (7A right panel and quantitation in 7C) following treatment with VX15/2503 antibody ("Anti-SEMA4D") or isotype control ("Control IgG").

[0019] FIGURE 8: Immunoblot results showing the effect of increasing concentrations of recombinant SEMA4D (1ng/ml, 10ng/ml and 100ng/ml) on the expression of the key endothelial tight junction protein Claudin-5 ("CLN-5") compared to VEGF-A positive control in primary mouse central nervous system (CNS) endothelial cultures.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0020] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an anti-SEMA4D antibody" is understood to represent one or more anti-SEMA4D antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0021] It should be noted that the term "blood brain barrier" and "BBB" are used interchangeably.

[0022] As used herein, the term "breakdown" or "disruption" with regards to the BBB, such as "blood brain barrier breakdown", "blood brain barrier disruption", "breakdown of the blood brain barrier", or "disruption of the blood brain barrier" refers to an increase in permeability of the blood brain barrier, or, in the case of the "DIV-BBB," a humanized dynamic *in vitro* model of BBB, a decrease in transendothelial electrical resistance (TEER). McCallister *et al., Brain Res.* 904:20–30 (2001); Santaguida *et al., Brain Res.*

1109:1–13 (2006); and Cucullo *et al., Epilepsia* 48:505–16 (2007) have shown that there is a direct (inverse) relationship between TEER and permeability in DIV-BBB. In addition, an increase in the permeability of the blood brain barrier or a decrease in electrical resistance can be the result of a decrease in the number, density and/or concentration of endothelial cells present on the BBB; or a change in the morphology or interactions among endothelial cells or astrocytes or between endothelial cells and astrocytes that form the BBB.

[0023] As used herein, the term "restoration" with regards to the BBB, such as "blood brain barrier restoration" or "restoration of the blood brain barrier" refers to a decrease in permeability of the blood brain barrier, or, in the case of the DIV-BBB, a humanized dynamic *in vitro* model of BBB, an increase in transendothelial electrical resistance.

[0024] As used herein, the term "neuroinflammatory disorder" refers to a central nervous system (CNS) inflammatory disorder, a neurodegenerative disorder, an autoimmune disorder of the central nervous system, a myelin disorder or a disease that affects oligodendrocytes, or a post-trauma myelin disorder of the central nervous system. It should be noted that neuroinflammatory disorders are often also neurodegenerative disorders. However, it is possible for a neurodegenerative disorder to exist in the absence of obvious neuroinflammation. This is the case, for example, in late stage secondary progressive multiple sclerosis.

[0025] The term "therapeutically effective amount" refers to an amount of an antibody, polypeptide, polynucleotide, small organic molecule, or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of a neuroinflammatory disorder, the therapeutically effective amount of the drug can decrease the permeability of the BBB; reduce, retard or stop an increase in BBB permeability; inhibit, e.g., suppress, retard, prevent, stop, or reverse an increased permeability of the BBB; increase the number, density and/or concentration of endothelial cells present on the BBB; change in the morphology or function of endothelial cells; or a change in the interactions among endothelial cells or astrocytes or between endothelial cells and astrocytes that form the BBB; relieve to some extent one or more of the symptoms associated with an increased BBB permeability, e.g., neuroinflammatory disorders; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

[0026] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cure, slow down, lessen symptoms of, reverse, and/or halt progression of a diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0027] 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 humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, bears, and so on.

[0028] As used herein, phrases such as "a subject that would benefit from administration of an anti-SEMA4D antibody" and "an animal in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of an anti-SEMA4D antibody or other SEMA4D binding molecule used, *e.g.*, for detection of a SEMA4D polypeptide (*e.g.*, for a diagnostic procedure) and/or from treatment, *i.e.*, palliation or prevention of a disease, with an anti-SEMA4D antibody or other SEMA4D binding molecule.

[0029] A "binding molecule" or "antigen binding molecule" of the present invention refers in its broadest sense to a molecule that specifically binds an antigenic determinant. In one embodiment, the binding molecule specifically binds to SEMA4D, *e.g.*, to a transmembrane SEMA4D polypeptide of about 150 kDa or a soluble SEMA4D polypeptide of about 120 kDa (commonly referred to as sSEMA4D). In another embodiment, a binding molecule of the invention is an antibody or an antigen binding

fragment thereof. In another embodiment, a binding molecule of the invention comprises at least one heavy or light chain CDR of an antibody molecule. In another embodiment, a binding molecule of the invention comprises at least two CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the invention comprises at least three CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the invention comprises at least four CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the invention comprises at least five CDRs from one or more antibody molecules. In another embodiment, a binding molecule of the invention comprises at least six CDRs from one or more antibody molecules.

[0030] The present application is directed to a method of decreasing blood brain barrier permeability in a subject having a neuroinflammatory disorder (*e.g.*, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, epilepsy, Alzheimer's Disease, Parkinson's Disease, meningitis, brain edema, brain trauma, and stroke), comprising administering to the subject an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof.

[0031] As used herein, "anti-SEMA4D binding molecule" or "anti-PlexinB1 binding molecule" refers to an antibody, or antigen-binding fragment, variant, or derivative thereof. Unless specifically referring to full-sized antibodies such as naturally occurring antibodies, the term "anti-SEMA4D antibody" or "anti-PlexinB1 antibody" encompasses full-sized antibodies as well as antigen-binding fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, naturally occurring antibody or immunoglobulin molecules or engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules.

[0032] As used herein, "inhibitor of SEMA4D interaction with a SEMA4D receptor" refers to an "anti-SEMA4D binding molecule", an "anti-PlexinB1 binding molecule" as well as a small molecule inhibitor of SEMA4D or a SEMA4D receptor.

[0033] As used herein, "human" or "fully human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described infra and, for example, in U.S. Pat. No. 5,939,598 by Kucherlapati *et al.* "Human" or

"fully human" antibodies also include antibodies comprising at least the variable domain of a heavy chain, or at least the variable domains of a heavy chain and a light chain, where the variable domain(s) have the amino acid sequence of human immunoglobulin variable domain(s).

[0034] "Human" or "fully human" antibodies also include "human" or "fully human" antibodies, as described above, that comprise, consist essentially of, or consist of, variants (including derivatives) of antibody molecules (*e.g.*, the VH regions and/or VL regions) described herein, which antibodies or fragments thereof immunospecifically bind to a SEMA4D polypeptide or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a human anti-SEMA4D antibody, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH region, VHCDR1, VHCDR2, VHCDR3, VL region, VLCDR1, VLCDR2, or VLCDR3.

[0035] In certain embodiments, the amino acid substitutions are conservative amino acid substitution, discussed further below. Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind a SEMA4D polypeptide, *e.g.*, human, murine, or both human and murine SEMA4D). Such variants (or derivatives thereof) of "human" or "fully human" antibodies can also be referred to as human or fully human antibodies that are "optimized" or "optimized for antigen binding" and include antibodies that have improved affinity to antigen.

[0036] The terms "antibody" and "immunoglobulin" are used interchangeably herein. An antibody or immunoglobulin comprises at least the variable domain of a heavy chain, and normally comprises at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood.

See, *e.g.*, Harlow et al. (1988) Antibodies: A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press).

[0037] As used herein, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention. All immunoglobulin classes are clearly within the scope of the present invention, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0038] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0039] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL or VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor

binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0040] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs) within these variable domains, of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three CDRs on each of the VH and VL chains. In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. See, *e.g.*, Hamers-Casterman *et al.*, *Nature 363*:446-448 (1993).

[0041] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable domain by one of ordinary skill in the art, since they have been precisely defined (see below).

[0042] In the case where there are two or more definitions of a term that is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat et al. (1983) U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" and by Chothia and Lesk, *J. Mol. Biol. 196*:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues that encompass the CDRs as defined by each of the above cited references are set forth below in **Table 1** as a comparison. The exact residue numbers that encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1. CDR Definitions[1]

	Kabat	Chothia
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

[0043] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.* (1983) U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest." Unless otherwise specified, references to the numbering of specific amino acid residue positions in an anti-SEMA4D

antibody or antigen-binding fragment, variant, or derivative thereof of the present invention are according to the Kabat numbering system.

[0044] Antibodies or antigen-binding fragments, variants, or derivatives thereof of the invention include, but are not limited to, polyclonal, monoclonal, multispecific and bispecific in which at least one arm is specific for SEMA4D, human, humanized, primatized, or chimeric antibodies, single-chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to anti-SEMA4D antibodies disclosed herein). ScFv molecules are known in the art and are described, *e.g.*, in U.S. Pat. No. 5,892,019. Immunoglobulin or antibody molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2, etc.), or subclass of immunoglobulin molecule.

[0045] As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. In certain embodiments, a polypeptide comprising a heavy chain portion comprises at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, a binding polypeptide for use in the invention may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the invention comprises a polypeptide chain comprising a CH3 domain. Further, a binding polypeptide for use in the invention may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0046] In certain anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein, the heavy chain portions of one polypeptide chain of a multimer are identical to those on a second polypeptide chain of the multimer. Alternatively, heavy

chain portion-containing monomers of the invention are not identical. For example, each monomer may comprise a different target binding site, forming, for example, a bispecific antibody.

[0047] The heavy chain portions of a binding molecule for use in the methods disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide can comprise a C_{H1} domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0048] As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain, *e.g.*, a kappa or lambda light chain. Preferably, the light chain portion comprises at least one of a VL or CL domain.

[0049] Anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein may be described or specified in terms of the epitope(s) or portion(s) of an antigen, *e.g.*, a target polypeptide disclosed herein (*e.g.*, SEMA4D) that they recognize or specifically bind. The portion of a target polypeptide that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target polypeptide can comprise a single epitope, but typically comprises at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen. Furthermore, it should be noted that an "epitope" on a target polypeptide may be or may include non-polypeptide elements, *e.g.*, an epitope may include a carbohydrate side chain.

[0050] The minimum size of a peptide or polypeptide epitope for an antibody is thought to be about four to five amino acids. Peptide or polypeptide epitopes preferably contain at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids. Since a CDR can recognize an antigenic peptide or polypeptide in its tertiary form, the amino acids comprising an epitope need not be contiguous, and in some cases, may not even be on the same peptide chain. A peptide or polypeptide epitope recognized by anti-SEMA4D antibodies of the present invention may contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least

10, at least 15, at least 20, at least 25, or between about 15 to about 30 contiguous or non-contiguous amino acids of SEMA4D.

[0051] By "specifically binds," it is generally meant that an antibody binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0052] By "preferentially binds," it is meant that the antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody that "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody may cross-react with the related epitope.

[0053] By way of non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds said first epitope with a dissociation constant (K_D) that is less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second epitope.

[0054] In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an off rate (k(off)) that is less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's k(off) for the second epitope. An antibody or

antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a target polypeptide disclosed herein (*e.g.,* SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1} or 10^{-3} sec^{-1}. More preferably, an antibody of the invention may be said to bind a target polypeptide disclosed herein (*e.g.,* SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an off rate (k(off)) less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1}, 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0055] An antibody or antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a target polypeptide disclosed herein (*e.g.,* SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an on rate (k(on)) of greater than or equal to 10^{3} M^{-1} sec^{-1}, 5 X 10^{3} M^{-1} sec^{-1}, 10^{4} M^{-1} sec^{-1} or 5 X 10^{4} M^{-1} sec^{-1}. More preferably, an antibody of the invention may be said to bind a target polypeptide disclosed herein (*e.g.,* SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D) or a fragment or variant thereof with an on rate (k(on)) greater than or equal to 10^{5} M^{-1} sec^{-1}, 5 X 10^{5} M^{-1} sec^{-1}, 10^{6} M^{-1} sec^{-1}, or 5 X 10^{6} M^{-1} sec^{-1} or 10^{7} M^{-1} sec^{-1}.

[0056] An antibody is said to competitively inhibit binding of a reference antibody to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0057] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with the CDR of an immunoglobulin molecule. See, *e.g.*, Harlow *et al.* (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2nd ed.) pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen. See, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent

monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity.

[0058] Anti-SEMA4D antibodies or antigen-binding fragments, variants, or derivatives thereof of the invention may also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of an antibody, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, an antibody is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, may actually fit better than the original.

[0059] For example, certain antibodies have some degree of cross-reactivity, in that they bind related, but non-identical epitopes, *e.g.*, epitopes with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be said to have little or no cross-reactivity if it does not bind epitopes with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be deemed "highly specific" for a certain epitope, if it does not bind any other analog, ortholog, or homolog of that epitope.

[0060] Anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof, of the invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention, *e.g.*, SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M. In certain embodiments, the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen binding fragment thereof, of the invention binds human SEMA4D with a Kd of about 5×10^{-9} to about 6×10^{-9}. In another embodiment, the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen

binding fragment thereof, of the invention binds murine SEMA4D with a Kd of about 1 x 10^{-9} to about 2 x 10^{-9}.

[0061] As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant invention) is obtained from a second species. In preferred embodiments the target binding region or site will be from a non-human source (*e.g.*, mouse or primate) and the constant region is human.

[0062] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy or light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable domain to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site.

[0063] It is further recognized that the framework regions within the variable domain in a heavy or light chain, or both, of a humanized antibody may comprise solely residues of human origin, in which case these framework regions of the humanized antibody are referred to as "fully human framework regions" (for example, MAb VX15/2503, disclosed in U.S. Patent Appl. Publication No. US 2010/0285036 A1 as MAb 2503, incorporated herein by reference in its entirety). Alternatively, one or more residues of the framework region(s) of the donor variable domain can be engineered within the corresponding position of the human framework region(s) of a variable domain in a heavy or light chain, or both, of a humanized antibody if necessary to maintain proper binding or to enhance binding to the SEMA4D antigen. A human framework region that has been engineered in this manner

would thus comprise a mixture of human and donor framework residues, and is referred to herein as a "partially human framework region."

[0064] For example, humanization of an anti-SEMA4D antibody can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature 321*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-327 (1988); Verhoeyen *et al.*, *Science 239*:1534-1536 (1988)), by substituting rodent or mutant rodent CDRs or CDR sequences for the corresponding sequences of a human anti-SEMA4D antibody. See also U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205; herein incorporated by reference. The resulting humanized anti-SEMA4D antibody would comprise at least one rodent or mutant rodent CDR within the fully human framework regions of the variable domain of the heavy and/or light chain of the humanized antibody. In some instances, residues within the framework regions of one or more variable domains of the humanized anti-SEMA4D antibody are replaced by corresponding non-human (for example, rodent) residues (see, for example, U.S. Pat. Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370), in which case the resulting humanized anti-SEMA4D antibody would comprise partially human framework regions within the variable domain of the heavy and/or light chain.

[0065] Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (*e.g.*, to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones *et al.*, *Nature 331*:522-525 (1986); Riechmann *et al.*, *Nature 332*:323-329 (1988); and Presta, *Curr. Op. Struct. Biol. 2*:593-596 (1992); herein incorporated by reference. Accordingly, such "humanized" antibodies may include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by residues from

analogous sites in rodent antibodies. See, for example, U.S. Pat. Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Pat. No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.

II. Blood Brain Barrier ("BBB")

[0066] The blood-brain barrier (BBB) is an active interface between circulating blood and the central nervous system (CNS). The BBB restricts the free movement of different substances between the two compartments and plays a crucial role in the maintenance of the homeostasis of the CNS. The BBB has both a barrier function and a carrier function. As a barrier, the BBB restricts the movement of cells and potentially toxic or harmful substances from the blood to the brain. As a carrier, on the other hand, the BBB is responsible for transporting nutrients to the brain and removing metabolites.

[0067] The BBB is primarily composed of three components: endothelial cells, astrocytes, and pericytes. Endothelial cells form a continuous sheet covering the inner surface of the capillaries and blood vessels in the brain. (Ransohoff et al., "Three or More Routes for Leukocyte Migration Into the Central Nervous System," *Nature Rev. Immun.* 3:569-581 (2003). The endothelial cells are located adjacent to the basal membrane, which consists mainly of collagen IV, fibronectin, laminin and proteoglycans, and are interconnected by tight junctions that form a belt-like structure at the apical region of the cells. Endothelial cells restrict the diffusion of microscopic objects (e.g. bacteria) and large or hydrophilic molecules into the brain parenchyma and cerebrospinal fluid (CSF), while allowing the diffusion of small hydrophobic molecules (O_2, hormones, CO_2). Cells of the barrier actively transport metabolic products such as glucose across the barrier with specific proteins.

[0068] The endothelial cells which form the brain capillaries are different from those found in other tissues in the body. Brain capillary endothelial cells are joined together by tight intercellular junctions that form a continuous wall against the passive diffusion of molecules from the blood to the brain and other parts of the CNS (including Cerebrospinal Fluid, CSF). These cells are also different in that they have few pinocytic vesicles which in other tissues allow somewhat unselective transport across the capillary

wall. Also lacking are continuous gaps or channels running between the cells which would allow unrestricted passage.

[0069] In addition to endothelial cells, the BBB is also composed of pericytes and astrocytes. Pericytes are located within the basal membrane, interact with endothelial cells and play an important role in the regulation of endothelial proliferation, angiogenesis and inflammatory processes. Astrocytes are characteristic star-shaped glial cells in the brain and spinal cord and are the most abundant cell of the human brain. They perform many functions, including biochemical support of endothelial cells that form the blood–brain barrier, provision of nutrients to the nervous tissue, maintenance of extracellular ion balance, and a role in the repair and scarring process of the brain and spinal cord following traumatic injuries.

[0070] The blood-brain barrier functions to ensure that the environment of the brain is constantly controlled. The levels of various substances in the blood, such as hormones, amino acids, and ions, undergo frequent small fluctuations which can be brought about by activities such as eating and exercise (Goldstein et al., "The Blood-Brain Barrier," Scientific American 255:74-83 (1986); Pardridge, "Receptor-Mediated Peptide Transport Through the Blood-Brain Barrier," Endocrin. Rev. 7:314-330 (1986)). If the brain was not protected by the blood brain barrier from these variations in serum composition, the result could be uncontrolled neural activity.

[0071] The isolation of the brain from the bloodstream is not complete. If this were the case, the brain would be unable to function properly due to a lack of nutrients and because of the need to exchange chemicals with the rest of the body. The presence of specific transport systems within the capillary endothelial cells assures that the brain receives, in a controlled manner, all of the compounds required for normal growth and function. In many instances, these transport systems consist of membrane-associated proteins, which selectively bind and transport certain molecules across the barrier membranes. These transporter proteins are known as solute carrier transporters.

[0072] Although the BBB serves to protect the brain and the central nervous system from damage from foreign or external molecules and cells, foreign or external molecules and cells can often cross the BBB and, in limited numbers, may even be beneficial such as for immune surveillance of the CNS. However, when highly active cells, such as, for instance, B cells, T cells, leukocytes and macrophages, cross the BBB in excess and reach

the brain, they can cause damage to the brain. Patients suffering from edema, brain traumas, stroke and multiple sclerosis, for instance, exhibit a breakdown of the BBB.

[0073] The effect of the BBB on various neuroinflammatory disorders has been studied. (Zlokovic BV, "The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders," Neuron 57: 178-201 (2008); Zhong Z et al., "ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration," Nature Neuroscience 11(4): 420-422 (2008); Hawkins BT et al., "The Blood-Brain Barrier/neurovascular Unit in Health and Disease," Pharmacological Rev 57 (2): 173-185 (2005); Oby E et al., "The Blood-Brain Barrier and Epilepsy," Epilepsia 47(11); 1761-1774 (2006)). Moreover, there is increasing evidence that inflammation and the blood–brain barrier (BBB) (Banks and Erickson, 2010; Lochhead et al, 2010) are involved in the pathogenesis of neurologic diseases such as meningitis (van der et al, 2004), brain edema (Stamatovic et al, 2006), Alzheimer's disease (Kalaria, 1992), Parkinson's disease (Westin, J.E., et. al., "Endothelial Proliferation and Increased Blood-Brain Barrier Permeability in the Basal Ganglia in a Rat Model of 3,4-Dihydroxyphenyl-L-Alanine-Induced Dyskinesia," The Journal of Neuroscience 26(37): 9448-9461 (2006)) and multiple sclerosis (Minagar and Alexander, 2003).

[0074] In the case of multiple sclerosis, for instance, it has been shown using Magnetic Resonance Imaging ("MRI"), that when a person is undergoing an MS "attack," the BBB has broken down in a section of the brain or spinal cord, allowing T lymphocytes to cross over and attack the myelin that protects and insulates the neurons of the central nervous systems in both brain and spinal cord. (Zlokovic 2008; Waubant E., "Biomarkers indicative of blood–brain barrier disruption in multiple sclerosis". Disease Markers 22 (4): 235–44 (2006)).

[0075] Meningitis, on the other hand, occurs when there is an inflammation of the membranes that surround the brain and spinal cord (these membranes are known as meninges). When the meninges are inflamed, the blood–brain barrier may be disrupted, allowing both inflammatory cells and various substances (including either toxins or antibiotics) to enter the brain. (Beam, TR Jr., et al. (December 1977). "Blood, brain, and cerebrospinal fluid concentrations of several antibiotics in rabbits with intact and inflamed meninges". Antimicrobial Agents and Chemotherapy 12 (6): 710–6).

[0076] Similarly, in the case of Parkinson's Disease (PD), it has been suggested that absorption or metabolism of putative PD toxins, and their faulty elimination across the BBB, due to low activity of the transporter P-glycoportein (P-gp), an ATP-dependent efflux pump which mediates rapid removal of ingested toxic lipophilic metabolites, may play a role in the pathogenesis of PD (Kortekaas, R., Leenders, K.L., van Oostrom, J.C., Vaalburg, W., Bart, J., Willemsen, A.T., and Hendrikse, N.H. *Blood-brain barrier dysfunction in parkinsonian midbrain in vivo.* Ann. Neurol. 57, 176–179, 2005). Neuroinflammation also appears to be a ubiquitous finding in PD patients and experimental models of PD. Phagocyte activation, increased synthesis and release of proinflammatory cytokines, complement activation, activation of microglia, and release of reactive oxygen species (ROS) have been described (Whitton, P.S. *Inflammation as a causative factor in the aetiology of Parkinson's disease.* Br. J. Pharmacol. 150, 963–976, 2007).

[0077] In epilepsy, studies have implicated the failure of blood–brain barrier function in triggering chronic or acute seizures due to certain interactions between a common blood protein, albumin, and astrocytes. These findings suggest that acute seizures are a result of disruption of the BBB by either artificial or inflammatory mechanisms. (Oby, E; et al. (2006). "The Blood–Brain Barrier and Epilepsy" (PDF). Epilepsia 47 (11): 1761–1774).

[0078] In patients with Alzheimer's Disease (AD), evidence points to the disruption of the blood–brain barrier in allowing blood plasma containing amyloid beta (Aβ) to enter the brain though RAGE, a major influx transporter for Aβ across the BBB. Studies have shown that the Aβ/RAGE interaction results in transcytosis of circulating Aβ across the BBB into the brain parenchyma and its binding to neurons, NF-kB-mediated endothelial activation resulting in secretion of proinflammatory cytokines, the expression of adhesion molecules, and the generation of endothelin-1, which suppresses CBF (Cerebral Blood Flow). Moreover, it has been shown that the Aβ/RAGE interaction contributes to neuronal killing by producing oxidative damage to RAGE-expressing neurons and by activating microglia. (Zlokovic, B.V. The Blood-Brain Barrier in Health and Chronic Neurodegenerative Disorders. Neuron 57, 178-201, 2008). Faulty efflux of Aβ out of the brain parenchyma and into the microvasculature via the BBB has also been found in the setting of AD pathogenesis and has been attributed, in part, to compromised low-density lipoprotein receptor related protein 1 (LRP1) function. LRP1 is an abluminal BBB membrane protein that binds and transports different structural conformers of Aβ (Deane

et al., "LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms." Neuron 43, 333–344, 2004). Aβ exposure shifts cell surface expression patterns of tight junction proteins, including claudin-5 and ZO-2, on brain microvascular endothelial cells to the cytoplasm (Marco et al., "Amyloid β-peptide 1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells." Neurosci. Lett. 401, 219-224, 2006), and severely compromises transendothelial electrical resistance (TEER) of monolayers of these cells (Gonzalez-Velasquez et al., "Soluble aggregates of the amyloid-beta protein selectively stimulate permeability in human brain microvascular endothelial monolayers." J. Neurochem. 107, 466-477, 2008).

[0079] In Amyotrophic lateral sclerosis (ALS), studies have suggested that BBB breakdown may result in leakage of serum proteins that interact with motor neurons to produce ROS (Reactive Oxygen Species) and initiate an autoimmune response, causing demyelination, disruption of neuronal transmission, and cell death. (Zlokovic 2008).

[0080] A recent study suggests that the weakening of the BBB can result from a disturbance in endothelial cells mediated through their VEGF-A receptor. (Argaw AT et al., "VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown," PNAS 106(6): 1977-1982 (2009)). According to that study, VEGF-A, which is derived from astrocytes, targets and disrupts expression of both endothelial transmembrane tight junction proteins claudin-5 (CLN-5) and occludin (OCLN). As expression of both CLN-5 and OCLN decreases, breakdown of the BBB increases.

[0081] As shown in the present examples, another possible mechanism for the weakening of the BBB is as a result of endothelial cell disturbance through the Plexin-B1 high affinity (1 nM) receptor for SEMA4D. Plexin-B1 can be expressed by endothelial cells. In the presence of SEMA4-D, endothelial cells may undergo a transformation which alters the morphology or function of the endothelial cells so as to cause a weakening of the BBB, for example, through modification of tight junctions. This weakening of the BBB may, in turn, increase permeability of the BBB to cells and molecules and allow such cells and molecules to enter and alter activity of the brain and central nervous system. Addition of either anti-SEMA4D or anti-Plexin-B1, consequently, may prevent endothelial cells from undergoing a transformation and reduce weakening of the BBB.

III. Target Polypeptide Description

[0082] As used herein, the terms "semaphorin-4D," "SEMA4D" and "SEMA4D polypeptide" are used interchangeably, as are "SEMA4D" and "Sema4D." In certain embodiments, SEMA4D is expressed on the surface of or secreted by a cell. In another embodiment, SEMA4D is membrane bound. In another embodiments, SEMA4D is soluble, *e.g.*, sSEMA4D. In other embodiments, SEMA4D may include a full-sized SEMA4D or a fragment thereof, or a SEMA4D variant polypeptide, wherein the fragment of SEMA4D or SEMA4D variant polypeptide retains some or all functional properties of the full-sized SEMA4D.

[0083] The full-sized human SEMA4D protein is a homodimeric transmembrane protein consisting of two polypeptide chains of 150 kDa. SEMA4D belongs to the semaphorin family of cell surface receptors and is also referred to as CD100. Both human and mouse SEMA4D/Sema4D are proteolytically cleaved from their transmembrane form to generate 120-kDa soluble forms, indicating the existence of two Sema4D isoforms (Kumanogoh *et al.*, *J. Cell Science 116*(7):3464 (2003)). Semaphorins include soluble and membrane-bound proteins that were originally defined as axonal-guidance factors during development which play an important role in establishing precise connections between neurons and their appropriate target. Structurally considered a class IV semaphorin, full-sized SEMA4D includes an amino-terminal signal sequence followed by a characteristic 'Sema' domain, which contains 17 conserved cysteine residues, an Ig-like domain, a lysine-rich stretch, a hydrophobic transmembrane region, and a cytoplasmic tail.

[0084] Each polypeptide chain of SEMA4D includes a signal sequence of about 13 amino acids followed by a semaphorin domain of about 512 amino acids, an immunoglobulin-like (Ig-like) domain of about 65 amino acids, a lysine-rich stretch of 104 amino acids, a hydrophobic transmembrane region of about 19 amino acids, and a cytoplasmic tail of 110 amino acids. A consensus site for tyrosine phosphorylation in the cytoplasmic tail supports the predicted association of SEMA4D with a tyrosine kinase (Schlossman, et al., Eds. (1995) Leucocyte Typing V (Oxford University Press, Oxford)).

[0085] SEMA4D is known to have at least two receptors. One of the receptors, Plexin-B1, is expressed in non-lymphoid tissues and has been shown to be a high affinity (1 nM) receptor for SEMA4D (Tamagnone *et al.*, *Cell 99*:71-80 (1999)). SEMA4D stimulation

of Plexin-B1 signaling has been shown to induce growth cone collapse of neurons, and to induce process extension collapse and apoptosis of oligodendrocytes (Giraudon *et al.*, *J. Immunol. 172*:1246-1255 (2004); Giraudon *et al.*, *NeuroMolecular Med. 7*:207-216 (2005)). After binding to SEMA4D, Plexin-B1 signaling mediates the inactivation of R-Ras, leading to a decrease in the integrin mediated attachment to the extracellular matrix, as well as to activation of RhoA, leading to reorganization of the cytoskeleton and cell migration. See Kruger *et al.*, *Nature Rev. Mol. Cell Biol. 6*:789-800 (2005); Pasterkamp, *TRENDS in Cell Biology 15*:61-64 (2005)).

[0086] In lymphoid tissues CD72 is utilized as a low affinity (300nM) SEMA4D receptor (Kumanogoh *et al.*, *Immunity 13*:621-631 (2000)). B cells and APCs express CD72, and anti-CD72 antibodies have many of the same effects as sSEMA4D, such as enhancement of CD40-induced B cell responses and B cell shedding of CD23. CD72 is thought to act as a negative regulator of B cell responses by recruiting the tyrosine phosphatase SHP-1, which can associate with many inhibitory receptors. Interaction of SEMA4D with CD72 results in the dissociation of SHP-1, and the loss of this negative activation signal. SEMA4D has been shown to promote T cell stimulation and B cell aggregation and survival *in vitro*. The addition of SEMA4D-expressing cells or sSEMA4D enhances CD40-induced B cell proliferation and immunoglobulin production *in vitro*, and accelerates *in vivo* antibody responses (Ishida *et al.*, *Inter. Immunol. 15*:1027-1034 (2003); Kumanogoh and H. Kukutani, *Trends in Immunol. 22*:670-676 (2001)). sSEMA4D enhances the CD40 induced maturation of DCs, including up-regulation of costimulatory molecules and increased secretion of IL-12. In addition, sSEMA4D can inhibit immune cell migration, which can be reversed by addition of blocking anti-SEMA4D antibodies (Elhabazi *et al.*, *J. Immunol.* 166:4341-4347 (2001); Delaire *et al.*, *J. Immunol.* 166:4348-4354 (2001)).

[0087] Sema4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, Sema4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). Cellular activation increases the surface expression of SEMA4D as well as the generation of soluble SEMA4D (sSEMA4D).

[0088] The expression pattern of SEMA4D suggests that it plays an important physiological role as well as pathological role in the immune system. SEMA4D has been shown to promote B cell activation, aggregation and survival; enhance CD40-induced proliferation and antibody production; enhance antibody response to T cell dependent antigens; increase T cell proliferation; enhance dendritic cell maturation and ability to stimulate T cells; and is directly implicated in demyelination and axonal degeneration (Shi *et al.*, *Immunity 13*:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); and Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001)).

[0089] SEMA4D knock out (SEMA4D-/-) mice have provided additional evidence that SEMA4D plays an important role in both humoral and cellular immune responses. There are no known major abnormalities of non-lymphoid tissues in SEMA4D-/- mice. Dendritic cells (DCs) from the SEMA4D-/- mice have poor allostimulatory ability and show defects in expression of costimulatory molecules, which can be rescued by the addition of sSEMA4D. Mice deficient in SEMA4D (SEMA4D-/-) fail to develop experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein peptide, because myelin oligodendrocyte glycoprotein-specific T cells are poorly generated in the absence of SEMA4D (Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002)). A significant amount of soluble SEMA4D is also detected in the sera of autoimmunity-prone MRL/lpr mice (model of systemic autoimmune diseases such as SLE), but not in normal mice. Further, the levels of sSEMA4D correlate with levels of auto-antibodies and increase with age (Wang *et al.*, *Blood 97*:3498-3504 (2001)). Soluble SEMA4D has also been shown to accumulate in the cerebral spinal fluid and sera of patients with demyelinating disease, and sSEMA4D induces apoptosis of human pluripotent neural precursors (Dev cells), and both inhibits process extension and induces apoptosis of rat oligodendrocytes *in vitro* (Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004)). This apoptosis was blocked by an anti-SEMA4D MAb.

IV. Anti-SEMA4D Antibodies

[0090] Antibodies that bind SEMA4D have been described in the art. See, for example, US Publ. Nos. 2008/0219971 A1, US 2010/0285036 A1, and US 2006/0233793 A1, International Patent Applications WO 93/14125, WO 2008/100995, and WO 2010/129917, and Herold *et al.*, *Int. Immunol. 7*(1): 1-8 (1995), each of which is herein incorporated in its entirety by reference.

[0091] The present application generally relates to a method of decreasing blood brain barrier permeability in a subject, *e.g.*, a human patient, having a neuroinflammatory disorder, *e.g.*, a CNS inflammatory disorder or neurodegenerative disorder, comprising administration of an antibody which specifically binds to SEMA4D, or an antigen-binding fragment, variant, or derivative thereof. In certain embodiments, the antibody blocks the interaction of SEMA4D with one or more of its receptors, *e.g.*, Plexin-B1. Anti-SEMA4D antibodies having these properties can be used in the methods provided herein. Antibodies that can be used include, but are not limited to MAbs VX15/2503, 67, and 76 and antigen-binding fragments, variants, or derivatives thereof which are fully described in US 2010/0285036 A1. Additional antibodies which can be used in the methods provided herein include the BD16 and BB18 antibodies described in US 2006/0233793 A1 as well as antigen-binding fragments, variants, or derivatives thereof; or any of MAb 301, MAb 1893, MAb 657, MAb 1807, MAb 1656, MAb 1808, Mab 59, MAb 2191, MAb 2274, MAb 2275, MAb 2276, MAb 2277, MAb 2278, MAb 2279, MAb 2280, MAb 2281, MAb 2282, MAb 2283, MAb 2284, and MAb 2285, as well as any fragments, variants or derivatives thereof as described in US 2008/0219971 A1. In certain embodiments an anti-SEMA4D antibody for use in the methods provided herein binds human, murine, or both human and murine SEMA4D. Also useful are antibodies which bind to the same epitope as any of the aforementioned antibodies and/or antibodies which competitively inhibit any of the aforementioned antibodies from binding to SEMA4D.

[0092] In certain embodiments, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein has an amino acid sequence that has at least about 80%, about 85%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, or about 95% sequence identity to the amino acid sequence for a reference anti-SEMA4D antibody molecule, for example those described above. In a further embodiment, the binding molecule shares at least about 96%, about 97%, about 98%, about 99%, or 100% sequence identity to a reference antibody.

[0093] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence that is at least about

80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 9 or 10.

[0094] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

[0095] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain), where at least one of the CDRs of the VH domain has an amino acid sequence identical, except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

[0096] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of a VH domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 9 or SEQ ID NO: 10, wherein the anti-SEMA4D antibody comprising the encoded VH domain specifically, preferentially, or competitively binds to SEMA4D.

[0097] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 17 or 18.

[0098] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence that is at least about

80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.

[0099] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VL domain has an amino acid sequence identical, except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.

[0100] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of a VL domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 17 or SEQ ID NO: 18, wherein the anti-SEMA4D antibody comprising the encoded VL domain specifically, preferentially, or competitively binds to SEMA4D.

[0101] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain) and an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VH domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 9 or 10 and at least one of the CDRs of the VL domain has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or identical to CDR1, CDR2 or CDR3 of SEQ ID NO: 17 or 18.

[0102] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of an immunoglobulin heavy chain variable domain (VH domain) and an immunoglobulin light chain variable domain (VL domain), where at least one of the CDRs of the VH domain has an amino acid sequence identical, except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8 and where at least one of the CDRs of the VL domain has an amino acid sequence identical,

except for 1, 2, 3, 4, or 5 conservative amino acid substitutions, to SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 16.

[0103] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of a VH domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 9 or SEQ ID NO: 10, and a VL domain that has an amino acid sequence that is at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% identical to SEQ ID NO: 17 or SEQ ID NO: 18, wherein the anti-SEMA4D antibody comprising the encoded VH and VL domains specifically, preferentially, or competitively binds to SEMA4D.

[0104] In another embodiment, an anti-SEMA4D antibody or antigen-binding fragment, variant, or derivative thereof useful in the methods provided herein comprises, consists essentially of, or consists of the three CDRs of the VL domain and three CDRs of the VH domain of MAb VX15/2503, 67, or 76, which are fully described in US 2010/0285036 A1. In some embodiments, the anti-SEMA4D antibody useful in the methods provided herein comprises MAb VX15/2503 or 67.

[0105] Also included for use in the methods provided herein are polypeptides encoding anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof as described herein, polynucleotides encoding such polypeptides, vectors comprising such polynucleotides, and host cells comprising such vectors or polynucleotides, all for producing anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof for use in the methods described herein.

[0106] Suitable biologically active variants of the anti-SEMA4D antibodies of the invention can be used in the methods of the present invention. Such variants will retain the desired binding properties of the parent anti-SEMA4D antibody. Methods for making antibody variants are generally available in the art.

[0107] Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York); Kunkel, Proc. *Natl. Acad. Sci. USA 82*:488-492 (1985); Kunkel *et al.*, *Methods Enzymol. 154*:367-382 (1987); Sambrook *et*

al. (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, N.Y.); U.S. Pat. No. 4,873,192; and the references cited therein; herein incorporated by reference. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the polypeptide of interest may be found in the model of Dayhoff *et al.* (1978) in Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.), pp. 345-352, herein incorporated by reference in its entirety. The model of Dayhoff *et al.* uses the Point Accepted Mutation (PAM) amino acid similarity matrix (PAM 250 matrix) to determine suitable conservative amino acid substitutions. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. Examples of conservative amino acid substitutions as taught by the PAM 250 matrix of the Dayhoff *et al.* model include, but are not limited to, Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln, and Phe↔Trp↔Tyr.

[0108] In constructing variants of the anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof, polypeptides of interest, modifications are made such that variants continue to possess the desired properties, *e.g.*, being capable of specifically binding to a SEMA4D, *e.g.*, human, murine, or both human and murine SEMA4D, *e.g.*, expressed on the surface of or secreted by a cell and having SEMA4D blocking activity, as described herein. Obviously, any mutations made in the DNA encoding the variant polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See EP Patent Application Publication No. 75,444.

[0109] Methods for measuring anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, binding specificity include, but are not limited to, standard competitive binding assays, assays for monitoring immunoglobulin secretion by T cells or B cells, T cell proliferation assays, apoptosis assays, ELISA assays, and the like. See, for example, such assays disclosed in WO 93/14125; Shi *et al.*, *Immunity 13*:633-642 (2000); Kumanogoh *et al.*, *J Immunol 169*:1175-1181 (2002); Watanabe *et al.*, *J Immunol 167*:4321-4328 (2001); Wang *et al.*, *Blood 97*:3498-3504 (2001); and Giraudon *et al.*, *J Immunol 172*(2):1246-1255 (2004), all of which are herein incorporated by reference.

[0110] When discussed herein whether any particular polypeptide, including the constant regions, CDRs, VH domains, or VL domains disclosed herein, is at least about 65%,

about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or even about 100% identical to another polypeptide, the % identity 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, Wis. 53711). BESTFIT uses the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482-489, 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.

[0111] For purposes of the present invention, percent sequence identity may be determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) Adv. Appl. Math. 2:482-489. A variant may, for example, differ from a reference anti-SEMA4D antibody (*e.g.*, MAb VX15/2503, 67 or 76) by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0112] The constant region of an anti-SEMA4D antibody can be mutated to alter effector function in a number of ways. For example, see U.S. Pat. No. 6,737,056B1 and U.S. Patent Application Publication No. 2004/0132101A1, which disclose Fc mutations that optimize antibody binding to Fc receptors.

[0113] In certain anti-SEMA4D antibodies or fragments, variants or derivatives thereof useful in the methods provided herein, the Fc portion can be mutated to decrease effector function using techniques known in the art. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases, constant region modifications consistent with the instant invention moderate

complement binding and thus reduce the serum half-life. Yet other modifications of the constant region can be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. The resulting physiological profile, bioavailability and other biochemical effects of the modifications, such as tumor localization, biodistribution and serum half-life, can easily be measured and quantified using well known immunological techniques without undue experimentation.Anti-SEMA4D antibodies for use in the methods provided herein include derivatives that are modified, *e.g.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from specifically binding to its cognate epitope. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the derivative can contain one or more non-classical amino acids.

[0114] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families 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). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind an anti-SEMA4D polypeptide, to block SEMA4D interaction with its receptor, or to decrease BBB permeability in a subject, e.g., a patient with a neuroinflammatory disorder).

[0115] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations can be silent or neutral missense mutations, *i.e.*, have no, or little, effect on an antibody's ability to bind antigen. These types of mutations can be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (*e.g.*, ability to immunospecifically bind at least one epitope of a SEMA4D polypeptide) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0116] In certain embodiments, the anti-SEMA4D antibodies for use in the methods provided herein comprise at least one optimized complementarity-determining region (CDR). By "optimized CDR" is intended that the CDR has been modified and optimized to improve binding affinity and/or anti-SEMA4D activity that is imparted to an anti-SEMA4D antibody comprising the optimized CDR. "Anti-SEMA4D activity" or "SEMA4D blocking activity" can include activity which modulates one or more of the following activities associated with SEMA4D: B cell activation, aggregation and survival; CD40-induced proliferation and antibody production; antibody response to T cell dependent antigens; T cell or other immune cell proliferation; dendritic cell maturation; demyelination and axonal degeneration; apoptosis of pluripotent neural precursors and/or oligodendrocytes; induction of endothelial cell migration; inhibition of spontaneous monocyte migration; binding to cell surface Plexin-B1 or other receptor, or any other activity associated with soluble SEMA4D or SEMA4D that is expressed on the surface of SEMA4D+ cells. Anti-SEMA4D activity can also be attributed to a decrease in incidence or severity of diseases associated with SEMA4D expression or overexpression, including, but not necessarily limited to, neuroinflammatory diseases including central nervous system (CNS) and peripheral nervous system (PNS) inflammatory diseases.

[0117] Examples of optimized antibodies based on murine anti-SEMA4D MAbs BD16 and BB18, were described in US Publ. No. 2008/0219971 A1, International Patent

Application WO 93/14125 and Herold *et al.*, *Int. Immunol.* 7(1): 1-8 (1995), each of which are herein incorporated by reference in their entirety. The modifications may involve replacement of amino acid residues within the CDR such that an anti-SEMA4D antibody retains specificity for the SEMA4D antigen and has improved binding affinity and/or improved anti-SEMA4D activity.

V. Treatment Methods Using Therapeutic Anti-SEMA4D and Anti-PlexinB1 Antibodies

[0118] Methods of the invention are directed to the use of an inhibitor of SEMA4D interaction with a SEMA4D receptor, *e.g.*, anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies, including antigen-binding fragments, variants, and derivatives thereof, to decrease blood brain barrier permeability in a subject having a neuroinflammatory disorder. In certain embodiments, the neuroinflammatory disorder is, *e.g.*, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, epilepsy, Alzheimer's Disease, Parkinson's Disease, meningitis, brain edema, brain trauma, or stroke. In certain embodiments, the endothelial cells express a SEMA4D receptor; and in certain embodiments, the receptor is Plexin-B1. Although the following discussion refers to administration of an anti-SEMA4D antibody, an anti-PlexinB1 antibody, and combination thereof, the methods described herein are also applicable to the antigen-binding fragments, variants, and derivatives of these anti-SEMA4D or anti-PlexinB1 antibodies that retain the desired properties of the anti-SEMA4D or anti-PlexinB1 antibodies of the invention, *e.g.*, capable of specifically binding SEMA4D, *e.g.*, human, mouse, or human and mouse SEMA4D, having SEMA4D neutralizing activity, and/or blocking the interaction of SEMA-4D with its receptor, *e.g.*, Plexin-B1.

[0119] In one embodiment, treatment includes the application or administration of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, an antibody or antigen binding fragment thereof as described herein to a patient, where the patient has, or has the risk of developing a neuroinflammatory disorder. In another embodiment, treatment is also intended to include the application or administration of a pharmaceutical composition comprising the anti-SEMA4D binding molecule, the anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, an antibody or antigen binding fragment thereof to a patient, where the patient has, or has the risk of developing a neuroinflammatory disorder. It should be appreciated that due to the interaction of SEMA4D with a receptor on endothelial cells, the application or

administration of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof is expected to occur on the blood side of the blood brain barrier. By administering an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecules, or combinations thereof by a route that exposes it to the blood side, *e.g.* including, but not limited to, intravenous administration, the anti-SEMA4D binding molecule, the anti-PlexinB1 binding molecules, or combinations thereof will be permitted to inhibit the interaction of SEMA4D with the SEMA4D receptor that is expressed by the endothelial cells.

[0120] The anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies or binding fragments thereof as described herein are useful for the treatment of various neuroinflammatory disorders. In some embodiments, treatment of a neuroinflammatory disorder is intended to include a reduction, or decrease, in permeability of the BBB. In other embodiments, treatment of a neuroinflammatory disorder is intended to include an increase in the resistivity of the BBB. In other embodiments, treatment of a neuroinflammatory disorder is intended to include an increase in the number, density and/or concentration of endothelial cells present on the BBB. In other embodiments, treatment of a neuroinflammatory disorder is intended to include a change in the morphology or function or endothelial cells, or in the interactions among endothelial cells or astrocytes or between endothelial cells and astrocytes that form the BBB.

[0121] In one embodiment, the invention relates to the use of anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof, as a medicament, in particular for use in the treatment or prophylaxis of neuroinflammatory disorders to inhibit, reduce, prevent, or minimize a breakdown in the BBB, or an increase in the permeability of the BBB.

[0122] In accordance with the methods of the present invention, at least one anti-SEMA4D binding molecule or anti-PlexinB1 binding molecule, *e.g.*, an antibody or antigen binding fragment, variant, or deriviative thereof, as defined elsewhere herein can be used to promote a positive therapeutic response with respect to the neuroinflammatory disorder. A "positive therapeutic response" with respect to the neuroinflammatory disorder is intended to include an improvement in the disease in association with the anti-inflammatory activity, anti-apoptotic activity, or the like, of these antibodies, and/or an

improvement in the symptoms associated with the disease. That is, an anti-proliferative effect, the prevention of further proliferation of the SEMA4D-expressing cell, a reduction in the inflammatory response including but not limited to reduced secretion of inflammatory cytokines, adhesion molecules, proteases, immunoglobulins (in instances where the SEMA4D bearing cell is a B cell), combinations thereof, and the like, increased production of anti-inflammatory proteins, a reduction in the number of autoreactive cells, an increase in immune tolerance, inhibition of autoreactive cell survival, reduction in apoptosis, reduction in endothelial cell migration, increase in spontaneous monocyte migration, reduction in and/or a decrease in one or more symptoms mediated by stimulation of sSEMA4D or SEMA4D-expressing cells can be observed. Such positive therapeutic responses are not limited to the route of administration and may comprise administration to the donor, the donor tissue (such as for example organ perfusion), the host, any combination thereof, and the like. In particular, the methods provided herein are directed to inhibiting, preventing, reducing, alleviating, or lessening the development of a neuroinflammatory disorder in a patient. Thus, for example, an improvement in the disease may be characterized as an absence of clinically observable symptoms, a decrease in BBB permeability, an increase in the number, density or concentration of endothelial cells present on the BBB, a change in the morphology or function of the endothelial cells, or a change in the interactions among endothelial cells and pericytes or astrocytes or between endothelial cells, pericytes and astrocytes that form the BBB.

[0123] Changes in the permeability of the BBB can be measured using *in vitro* models. In certain embodiments, a dynamic *in vitro* DIV-BBB model can be employed. Cucullo et al. have presented a DIV-BBB model composed of normal adult human brain microvascular endothelial cells and human adult astrocytes to study how haemodynamic changes and systemic inflammation affect the integrity of the brain microvasculature. Specifically, this model uses a cartridge, or hollow tube, to represent the blood brain barrier with the interior of the cartridge representing the blood side of the blood brain barrier and the exterior of the cartridge representing the brain side of the blood brain barrier. The interior of the cartridge is lined with adult human brain microvascular endothelial cells and exterior is lined with human adult astrocytes. As a blood brain barrier modifying agent, such as SEMA4D, is introduced into the lumen of the cartridge, the electrical current between the interior and exterior of the tube is monitored using Transendothelial

Electrical Resistance Measurement, described below. One embodiment of this model has the novelty of having transcapillary microholes to enable transendothelial cell trafficking between the vascular and the parenchymal compartment. An in depth description of the *in vitro* DIV-BBB model and the derivation and culture of the human microvascular endothelial cells and adult astrocytes employed can be found in, for instance, Cucullo *et al., Brain* Research. 951 243-254 (2002); and Cucullo *et al.*, *Journal of Cerebral Blood Flow & Metabolism.* 2:767-77 (2011). It should be appreciated that people skilled in the art will recognize that other BBB models have been described and usefully employed for studies of the role of BBB in disease in the prior art and that the present disclosure should not be limited to any one particular model.

[0124] The permeability of the BBB can be monitored using Transendothelial Electrical Resistance Measurement (TEER). TEER is used to monitor the integrity of the BBB in real time, which has been shown to correlate with the permeability of the BBB. The TEER system uses electronic multiplexing to measure multiple cartridges in quick succession and assesses the integrity and viability of tissue culture bilayers rapidly and reliably (Cucullo *et al.,* 2002; Cucullo *et al.,* 2010; Santaguida *et al,* 2006). In operation, the system applies an excitation voltage (0.06V) across the excitation electrodes inserted in each cartridge in the luminal and extraluminal compartments. A microcontroller computes the resistivity and capacitance (per cm^2) of the barrier from physical parameters. The values of capacitance are calculated by comparison of the voltage and current waveforms. The delay from peak-to-peak of the two waveforms is proportional to the capacitance value, which is expressed as arch tension. The TEER can be measured from the initial setup throughout the course of each experiment.

[0125] The anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies or antigen binding fragments, variants, or derivatives thereof can be used in combination with at least one or more other treatments for neuroinflammatory disorders; where the additional therapy is administered prior to, during, or subsequent to the anti-SEMA4D binding molecule, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibody or antigen binding fragment, variant, or derivative thereof, therapy. Thus, where the combined therapies comprise administration of an anti-SEMA4D binding molecule, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, an antibody or antigen binding fragment, variant, or derivative thereof, in combination with

administration of another therapeutic agent, the methods of the invention encompass coadministration, using separate formulations or a single pharmaceutical formulation, with simultaneous or consecutive administration in either order.

VI. Pharmaceutical Compositions and Administration Methods

[0126] Methods of preparing and administering anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof to a subject in need thereof are well known to or are readily determined by those skilled in the art. The route of administration of the anti-SEMA4D binding molecule, the anti-PlexinB1 binding molecule, or combination thereof, *e.g*, antibody, or antigen-binding fragment, variant, or derivative thereof, can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. While all these forms of administration are clearly contemplated as being within the scope of the invention, an example of a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. A suitable pharmaceutical composition for injection can comprise a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), optionally a stabilizer agent (*e.g.* human albumin), etc. However, in other methods compatible with the teachings herein, anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.

[0127] As discussed herein, anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combination thereof, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof can be administered in a pharmaceutically effective amount for the *in vivo* treatment of neuroinflammatory disorders. In this regard, it will be appreciated that the disclosed binding molecules can be formulated so as to facilitate administration and promote stability of the active agent. In certain embodiments, pharmaceutical compositions in accordance with the present invention comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. For the purposes of the instant application, a pharmaceutically

effective amount of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof, shall be held to mean an amount sufficient to achieve effective binding to a target and to achieve a benefit, *e.g.*, to decrease the permeability of the BBB in a patient with a neuroinflammatory disorder.

[0128] The pharmaceutical compositions used in 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.

[0129] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include, *e.g.*, water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject invention, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

[0130] More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and will preferably be preserved against the contaminating action of microorganisms, such as

bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Suitable formulations for use in the therapeutic methods disclosed herein are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

[0131] Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0132] In any case, sterile injectable solutions can be prepared by incorporating an active compound (*e.g.*, an anti-SEMA4D antibody, or antigen-binding fragment, variant, or derivative thereof, by itself or in combination with other active agents) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparations for injections are processed, filled into containers such as ampoules, bags, bottles, syringes or vials, and sealed under aseptic conditions according to methods known in the art. Further, the preparations may be packaged and sold in the form of a kit. Such articles of manufacture can have labels or package inserts indicating that the associated compositions are useful for treating a subject suffering from, or predisposed to a disease or disorder.

[0133] Parenteral formulations can be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions can be administered at specific fixed or variable intervals, *e.g.*, once a day, or on an "as needed" basis.

[0134] Certain pharmaceutical compositions used in this invention can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can 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.

[0135] The amount of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody, or fragment, variant, or derivative thereof, to be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0136] In keeping with the scope of the present disclosure, anti-SEMA4D antibodies, or antigen-binding fragments, variants, or derivatives thereof can be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic effect. The anti-SEMA4D antibodies, or antigen-binding fragments, variants or derivatives thereof can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. Those skilled in the art will further appreciate that a cocktail comprising one or more species of anti-SEMA4D binding molecules, anti-PlexinB1 binding molecules, or combinations thereof, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof, of the invention can be used.

[0137] By "therapeutically effective dose or amount" or "effective amount" is intended an amount of anti-SEMA4D binding molecule, anti-PlexinB1 binding molecule, or

combination thereof, *e.g.*, antibody or antigen binding fragment, variant, or derivative thereof, that when administered brings about a positive therapeutic response with respect to treatment of a patient with a disease to be treated, e.g., a decrease in the permeability of the BBB, an increase in the resistivity of the BBB, an increase in the number, density or concentration of endothelial cells present on the BBB, a change in the morphology or function in the endothelial cells, or a change in the interactions among endothelial cells or astrocytes or between endothelial cells and astrocytes that form the BBB.

[0138] Therapeutically effective doses of the compositions of the present invention, for the decrease in BBB permeability vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain embodiments the patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0139] The amount of at least one anti-SEMA4D binding molecule, anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody or binding fragment, variant, or derivative thereof, to be administered is readily determined by one of ordinary skill in the art without undue experimentation given the disclosure of the present invention. Factors influencing the mode of administration and the respective amount of at least one anti-SEMA4D binding molecule, anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody, antigen-binding fragment, variant or derivative thereof include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of anti-SEMA4D binding molecule, anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody, or fragment, variant, or derivative thereof, to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

[0140] The invention also provides for the use of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody of the invention, or antigen-binding fragment, variant, or derivative thereof, in the manufacture of a medicament for treating a subject for treating a neuroinflammatory disorder, wherein the

medicament is used in a subject that has been pretreated with at least one other therapy. By "pretreated" or "pretreatment" is intended the subject has received one or more other therapies (*e.g.*, been treated with at least one other neuroinflammatory therapy) prior to receiving the medicament comprising the anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, antibody or antigen-binding fragment, variant, or derivative thereof. "Pretreated" or "pretreatment" includes subjects that have been treated with at least one other therapy within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or even within 1 day prior to initiation of treatment with the medicament comprising the anti-SEMA4D binding molecule, for example, the monoclonal antibody VX15/2503 disclosed herein, or antigen-binding fragment, variant, or derivative thereof. It is not necessary that the subject was a responder to pretreatment with the prior therapy or therapies. Thus, the subject that receives the medicament comprising the anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, or combination thereof, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof could have responded, or could have failed to respond, to pretreatment with the prior therapy, or to one or more of the prior therapies where pretreatment comprised multiple therapies.

[0141] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Sambrook et al., ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook et al., ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis et al. U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian

Cells, (Cold Spring Harbor Laboratory); Wu et al., eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel et al. (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0142] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood et al., eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites et al., eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

[0143] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett et al., eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden et al., (Elsevere, Amsterdam); Goldsby et al., eds. (2000) Kuby Immunnology (4th ed.; H. Freemand & Co.); Roitt et al. (2001) Immunology (6th ed.; London: Mosby); Abbas et al. (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlan); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall2003); Harlow and Lane (1988) Antibodies: A

Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0144] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0145] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

[0146] The following examples demonstrate the efficacy of anti-SEMA4D antibody (VX15/2503) in reducing or preventing the breakdown of the BBB, i.e., a decrease in permeability of the BBB, in an *in vitro* DIV-BBB model as well as in an *in vivo* EAE model. An *in vivo* Alzheimer's Disease model experiment is also disclose herein. An in depth description about the *in vitro* DIV-BBB model can be found in, for instance, Cucullo *et al., Brain* Research. 951 243-254 (2002); and Cucullo *et al., Journal of Cerebral Blood Flow & Metabolism.* 1-11 (2010). The *in vivo* EAE and Alzheimer's Disease models are disclosed, *e.g.*, in Miller *et al., Curr Protoc Immunol.* CHAPTER: Unit–15.1, 2007; Colton *et al., J Alzheimers Dis* 15:571–587, 2008 and Wilcock *et al.*, J. *Neuroscience*, 29:7957–7965, 2009, respectively.

Example 1: Testing the ability of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.*, VX15/2503, to restore the integrity of the BBB following SEMA4D-induced breakdown of the BBB in an *in vitro* DIV-BBB model

[0147] **Experimental Design.** A dynamic *in vitro* BBB ("DIV-BBB") model was performed to study the effect of recombinant human SEMA4D (huSEMA4D-his) and VX15/2503 (described in detail in US 2010/0285036 A1, incorporated herein by reference in its entirety) on the integrity of the BBB. Two DIV-BBB cartridges were tested in the model. The basic experimental design is shown in FIG. 1. Increasing concentrations of recombinant SEMA4D (rSEMA4D) were added into the lumen at 12 hour intervals, allowing for equilibration (approximately 12 hours/concentration). rSEMA4D was initially added into the lumen at a concentration of 0.05μg/ml at time 0. The concentration of rSEMA4D increased by 10-fold at each interval, for instance, 0.5μg/ml at 12 hours, 5.0μg/ml at 24 hours, and 50.0μg/ml at 36 hours. TEER measurements were

taken between each interval as a reflection of changes in the permeability of the BBB at varying concentrations of rSEMA4D. Following addition of the final dose of rSEMA4D at 50.0μg/ml at 36 hours, VX15/2503 was added into the lumen at a concentration of 250μg/ml at 48 hours. At 72 hours, 24 hours following the addition of VX15/2503, the permeability of the BBB was again measured.

[0148] Transendothelial Electrical Resistance Measurement (TEER) was used to monitor the integrity of the BBB in real time. As mentioned above, the TEER system uses electronic multiplexing to measure multiple cartridges in quick succession and assesses the integrity and viability of tissue culture bilayers rapidly and reliably (Cucullo *et al.,* 2002; Santaguida *et al,* 2006). In this dynamic *in vitro* model, the cartridges, or hollow tubes, were set up to represent the blood brain barrier with the interior of the cartridge representing the blood side of the blood brain barrier and the exterior of the cartridge representing the brain side of the blood brain barrier. The interior of the cartridge was lined with adult human brain microvascular endothelial cells and the exterior was lined with human adult astrocytes. As a blood brain barrier modifying agent, such as SEMA4D, was introduced into the lumen of the cartridge, the electrical current between the interior and exterior of the tube was monitored using TEER. In operation, the TEER system applies an excitation voltage (0.06V) across the excitation electrodes inserted in each cartridge in the luminal and extraluminal compartments. A microcontroller computes the resistivity and capacitance (per cm^2) of the barrier from physical parameters. The values of capacitance are calculated by comparison of the voltage and current waveforms. The delay from peak-to-peak of the two waveforms is proportional to the capacitance value, which is expressed as arch tension. The TEER was measured from the initial setup throughout the course of each experiment.

[0149] **rSEMA4D-induced Increase in Permeability of the BBB.** Following formation of the BBB, the effect of rSEMA4D on the integrity of the BBB was measured by adding increasing concentrations of recombinant SEMA4D (rSEMA4D) into the lumen of the two cartridges. rSEMA4D was initially added into the lumen at a concentration of 0.05μg/ml at time 0. The concentration of rSEMA4D was increased 10-fold at each 12 hour interval, for instance, 0.5μg/ml at 12 hours, 5μg/ml at 24 hours and 50.0μg/ml at 36 hours. TEER measurements were taken between and during each interval as a reflection of changes in the permeability of the BBB at varying concentrations of rSEMA4D.

Overall, permeability of the BBB remained relatively stable at 0.05μg/ml of rSEMA4D. Starting at 0.5μg/ml, increasing concentrations of rSEMA4D (i.e., 0.5μg/ml, 5μg/ml and 50μg/ml) resulted in decreased TEER measurement reflecting increased permeability of the endothelial cell layer. These results are shown in FIG. 2.

[0150] **Antibody-induced Decrease in Permeability of rSEMA4D Treated BBB.** To measure the effect of an anti-SEMA4D antibody on the BBB following exposure to escalating dosage of rSEMA4D, VX15/2503 was added at a concentration of 250μg/ml at 48 hours. TEER measurements were taken at 72 hours. Treatment with VX15/2503 resulted in an overall decrease in permeability (or increase in resistivity) of the BBB in the two cartridges. This decrease in permeability reflects restoration of the BBB. The results are shown in FIG. 2.

Example 2: Testing the ability of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.*, VX15/2503, to restore the integrity of the BBB following SEMA4D-induced breakdown of the BBB in an *in vitro* DIV-BBB model

[0151] **Experimental Design.** A second experiment employing the *in vitro* DIV-BBB model was performed to study the effect of SEMA4D and VX15/2503 on the integrity of the BBB. The basic experimental design was similar to that shown in Example 1, and FIG. 1, above. For two weeks, the DIV-BBB cartridges underwent BBB formation in endothelial and astrocytic cell compartments. The formation of the BBB as reflected in TEER is shown in FIGS. 3 and 4.

[0152] **rSEMA4D-induced Increase in Permeability of the BBB.** Following formation of the BBB, the effect of rSEMA4D on the integrity of the BBB was measured by adding increasing concentrations of recombinant SEMA4D (rSEMA4D) into the lumen of the first cartridge of a set of three cartridges at 12 hour intervals, allowing for equilibration (approximately 12 hours/concentration). rSEMA4D was initially added into the lumen at a concentration of 0.5μg/ml at time 0. The concentration of rSEMA4D increased by 10-fold at each interval, for instance, 5μg/ml at 12 hours and 50.0μg/ml at 24 hours. TEER measurements were taken between each interval as a reflection of changes in the permeability of the BBB at varying concentrations of rSEMA4D. Overall, increasing

concentrations of rSEMA4D resulted in decreased TEER measurement reflecting increased permeability of the BBB. These results are shown in FIG. 3.

[0153] To test the integrity of the BBB in the presence of an antigen that does not target the endothelial cell layer, a similarly prepared recombinant protein control (CTRL, C35 protein) was added at equimolar concentrations at the same 12 hour intervals (i.e., 0.25μg/ml at time 0, 2.5μg/ml at 12 hours, and 25.0μg/ml at 24 hours) to the two additional control cartridges. In contrast to the effect of rSEMA4D, the CTRL protein did not induce a significant change in TEER reflecting no meaningful change in permeability of the BBB. If, however, 50.0μg/ml of rSEMA4D was added 12 hours after addition of the highest concentration of CTRL protein, a rapid decrease in TEER similar to that observed with escalating doses of rSEMA4D was induced. The results are shown in FIG. 4.

[0154] **Antibody-induced Decrease in Permeability of rSEMA4D Treated BBB.** Following addition of the final dose of rSEMA4D at 50.0μg/ml at 24 hours, the effect of VX15/2503 on TEER and the permeability of the BBB was measured. In FIG. 3, VX15/2503 antibody was added at a concentration of 250μg/ml at 36 hours to two of the three cartridges that received escalating doses of rSEMA4D while the same concentration of an isotype control antibody was added to the one remaining cartridge that had received escalating doses of rSEMA4D. TEER measurements were taken at various subsequent points of time. Treatment with VX15/2503 resulted in an increase in TEER back to peak levels at the start of the experiment, reflecting an overall decrease in permeability of the BBB (i.e., restoration of the BBB). In the one cartridge that received isotype control antibody, TEER levels remained at the relatively reduced levels induced by treatment with rSEMA4D, indicating no meaningful decrease in permeability of the BBB. Similar results are shown in FIG. 4. In FIG. 4, VX15/2503 antibody was added at a concentration of 250μg/ml at 48 hours to the two cartridges that received initial control recombinant C35 protein followed by 50μg/ml of rSEMA4D for 12 hours. Treatment with VX15/2503 resulted in an increase in TEER back to peak levels at the start of the experiment, reflecting an overall decrease in permeability of the BBB (i.e., restoration of the BBB).

Example 3: Testing the ability of an anti-Plexin-B1 binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, to restore the integrity of the BBB following SEMA4D-induced breakdown of the BBB in an *in vitro* DIV-BBB model

[0155] Another study was conducted to measure the effects of anti-Plexin-B1 antibody (MAB37491 Human Plexin-B1 MAb (Clone 559830), R&D Systems) on the integrity of the BBB. This antibody blocks binding of SEMA4D to the Plexin-B1 receptor . The results of this study are shown in FIG. 5. As shown in FIG.5, human endothelial cells and astrocytes in four DIV-BBB cartridges underwent BBB formation similar to the experiments described above. After BBB formation, rSEMA4D was added at a concentration of 50.0μg/ml, inducing an increase in BBB permeability (i.e., destruction of the BBB). Following addition of rSEMA4D, anti-Plexin-B1 antibody was added at a concentration of 125μg/ml at 6 hours to two of the four cartridges, VX15/2503 antibody was added at a concentration of 250μg/ml to one of the four cartridges, and isotype control antibody was added at a concentration of 250μg/ml to the remaining cartridge. TEER measurements were taken at various subsequent points of time. Treatment with either VX15/2503 or anti-Plexin-B1 antibody resulted in an increase in TEER levels with both agents. Treatment with VX15/2503 resulted in a somewhat greater increase in TEER than treatment with anti-Plexin-B1 antibody at the last time point. The effect of the two antibodies is indistinguishable at all other time points. The increase in TEER reflects an overall decrease in permeability of the BBB (i.e., restoration of the BBB) in the presence of either VX15/2503 or anti-Plexin-B1 antibody. In the one cartridge that received isotype control antibody, TEER levels remained at the relatively reduced levels induced by treatment with rSEMA4D, indicating no meaningful decrease in permeability of the BBB. It should be appreciated that treatment can also be conducted using a combination of VX15/2503 and anti-Plexin-B1.

Example 4: Testing the ability of an anti-SEMA4D binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.*, VX15/2503, to restore the integrity of the BBB following breakdown of the BBB induced by activated PBMC and flow cessation in an *in vitro* DIV-BBB model

[0156] **Experimental Design.** Another experiment employing the *in vitro* DIV-BBB model was performed to study the effect of VX15/2503 on restoring the integrity of the BBB

following breakdown of the BBB induced by activated peripheral blood mononuclear cells (PBMC) and flow cessation. For two weeks, two DIV-BBB cartridges underwent BBB formation in endothelial and astrocytic cell compartments.

[0157] **Activated PBMC-induced Increase in Permeability of the BBB.** Following formation of the BBB, the effect of activated PBMC on the integrity of the BBB was measured. PBMC were activated with PMA/ionomycin for 2 hours and then added at a concentration of 10^6/ml into the lumen of the two cartridges. TEER measurements were taken prior to and after the addition of the activated PBMC as a reflection of changes in the permeability of the BBB. Overall, adding activated PBMC to the cartridges at 10^6/ml resulted in decreased TEER measurement reflecting increased permeability of the BBB. These results are shown in FIG. 6.

[0158] At approximately 2-4 hours following the addition of the activated PBMC to the cartridges, flow cessation was performed for 1 hour. TEER measurements were taken before and after flow cessation as a reflection of changes in the permeability of the BBB. Overall, flow cessation resulted in a further decrease in TEER measurement reflecting increased permeability of the BBB. These results are also shown in FIG. 6.

[0159] **Antibody-induced Decrease in Permeability of the BBB Exposed to Activated PBMC.** Following exposure to activated PBMC and flow cessation, the effect of VX15/2503 on TEER and the permeability of the BBB was measured. VX15/2503 antibody was added at a concentration of 250μg/ml to one of the two cartridges that received activated PBMC while the same concentration of an isotype control antibody (Isotype Control Ig, 2269) was added to the remaining cartridge. TEER measurements were taken at various subsequent points of time. As shown in FIG. 6, treatment with VX15/2503 resulted in an increase in TEER back to peak levels at the start of the experiment, reflecting an overall decrease in permeability of the BBB (i.e., restoration of the BBB). In the cartridge that received isotype control antibody, TEER levels remained at the relatively reduced levels induced by treatment with activated PBMC and flow cessation, indicating no meaningful decrease in the permeability of the BBB.

Example 5: Testing the ability of an anti-SEMA4D binding molecule, e.g., an antibody or antigen-binding fragment, variant, or derivative thereof, *e.g.,* VX15/2503, to protect the integrity of the BBB in an *in vivo* EAE model

[0160] Anti-SEMA4D binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof, *e.g.*, VX15/2503, were tested in the *in vivo* experimental autoimmune encephalomyelitis (EAE) model.

[0161] In an *in vivo* EAE model, the breakdown of the BBB was investigated by examining changes in brain permeability as reflected in the penetration of fibrinogen from blood into the brain parenchyma and through examination of endothelial tight junction proteins, including Claudin-5. In this model, EAE was induced in mice by immunization with PLP peptide (139-151). Of course, those skilled in the art will appreciate that other EAE inducing proteins may be used as well (e.g., a myelin antigen, for instance myelin-oligodendrocyte glycoprotein peptide 35-55) and that, for greatest efficiency, these inducing proteins or peptides may vary from one species to another and from one strain of mice to another, Steinman, L. *Neuron* 24:511-514 (1999). Tissue sections from the central nervous system (CNS) of animals at different stages of disease were then immunostained for proteins (fibrinogen and claudin-5, which serve as markers for BBB disruption).

[0162] **Experimental Design.** In an *in vivo* EAE model, EAE was induced in 12 week old SJL/J mice (10 mice per group) by immunization with PLP peptide (139-151) in CFA (complete Freund's adjuvant). The mice were then treated once per week from 7 days post-induction with 600μg anti-SEMA4D antibody (VX15/2503 antibody) or control IgG. Neurological signs were first observed at 11d postinduction (dpi). At 13 days postinduction, during the acute phase of disease, 4 mice per group were sacrificed and lumbar spinal cord samples were prepared for histopathologic analysis. To detect BBB disruption in the samples, these samples were immunostained for fibrinogen and claudin-5. The procedure for immunostaining is as follows: Sections were rinsed twice in PBS, then incubated in PBS 0.1% glycine 10 min, blocked in PBS 0.3% Triton X-100 10% goat serum for 1 h, and incubated with primary Abs in blocking buffer overnight at 4°C. For claudin-5 (CLN-5), prior to blocking, sections were soaked in EDTA, pH 8, 100°C. Primary antibodies used were anti-CLN-5 (1:50), and anti-fibrinogen (1:1,000). After washing three times in PBS 0.3%, Triton X-100 sections were then incubated in relevant species specific secondary antibodies conjugated to AlexaFluor 488 and/or AlexaFluor 594 (1/100; Molecular Probes) in blocking buffer for 1 h at 25°C, washed again three times, and counterstained with 4,6-diamidino-2-phenylindole (DAPI). All samples were

examined and photographed using a Zeiss LSM 510 META laser scanning confocal system attached to an Axiovert 200 inverted fluorescence microscope.

[0163] Clinical disease in the mice was scored as follows: 0= no symptoms; 1= floppy tail; 2= hind limb weakness; 4= fore and hind limb weakness; 5= death. Neurological signs were first observed at 11 days postinduction. In the mice treated with the VX15/2503 antibody, clinical disease reached a mean severity score of 0.75, indicative of mild tail weakness, while clinical disease in mice of the control group reached a mean severity scope of 2.25, indicative of paraparesis.

[0164] Results of the immunostaining at 13 days postinduction are shown in FIGS. 7A-7C. Fibrinogen does not normally penetrate the blood-brain brain barrier (BBB). In EAE, with the BBB compromised, the green fibrinogen stain was detected in brain matter (left panel). In addition, expression of claudin-5 (CLN-5, red stain), a component of the tight junctions that make up the BBB, was reduced. Mice in the control group showed reduced expression of claudin-5 and increased levels of extravascular leakage of fibrinogen, which correlated with a disruption in the BBB. In mice treated with VX15/2503 antibody, on the other hand, expression of claudin-5 was maintained and leakage of fibrinogen was significantly reduced. These results demonstrated the protective effect of VX15/2503 antibody against disruption of the BBB in these treated mice, and specifically demonstrated how anti-SEMA4D antibody prevents BBB breakdown, prevents extravascular leakage of fibrinogen (7A left panel and quantitation in 7B), and preserves claudin-5 as detected by red stain (7 A right panel and quantitation in 7C).

Example 6: Effect of SEMA4D on Tight Junction Proteins in cultures of cerebral endothelial cells

[0165] **Experimental Design.** The expression of the key endothelial tight junction protein Claudin-5 following treatment of CNS derived endothelial cells with soluble recombinant SEMA4D was investigated. In this model, primary mouse central nervous system (CNS) endothelial cultures were isolated and plated on a 6-well matrigel-coated plate (isolated MBCEC from 10 brains were resuspended in 3 ml primary endothelial cell culture medium and plated at 250 ul per well). Cultures were used at day 7 after isolation. Cultures were treated with 1ng/ml, 10ng/ml or 100ng/ml recombinant mouse SEMA4D or 100ng/ml mouse VEGF-A (positive control) for 24 hours. The endothelial cultures of the

animals were then subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting for the claudin-5 tight junction protein and actin loading control. Data were scanned and subjected to densitometry using ImageJ software (NIH).

[0166] Results of immunoblotting are shown in FIG. 8. As provided in FIG.8, endothelial cell cultures treated with 100ng/ml of recombinant SEMA4D showed a significant reduction in Claudin-5 protein expression. Endothelial cell cultures treated with 100ng/ml of VEGF-A were tested as a positive control for down-regulation of Claudin-5. This demonstrates the important role of SEMA4D in regulating expression of a key tight junction protein of the BBB.

Example 7: Testing the ability of an anti-SEMA4D or Anti-PlexinB1 binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative thereof to decrease the permeability of the BBB in an *in vivo* Alzheimer's disease (AD) model

[0167] Anti-SEMA4D or Anti-PlexinB1 binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof, *e.g.*, MAb 67 (described in detail in US 2010/0285036 A1, incorporated herein by reference in its entirety), are tested in various model systems of neuroinflammatory disorders, including, but not limited to an *in vivo* experimental Alzheimer's disease (AD) transgenic mouse model APPSwDI/NOSC$^{-/-}$. These mice were generated by crossing APP-Swedish-Dutch-Iowa mutant mouse with nitric oxide synthase 2 knock-out mouse (Colton *et al.*, *J Alzheimers Dis.*15:571–587, 2008; Van Nostrand *et al.*, *Stroke* 41:S135-S138, 2010). APPSwDI/NOSC$^{-/-}$ mice develop age-related neurovascular amyloidosis with disrupted BBB function, intraparenchymal amyloid plaques, mouse tau hyperphosphorylation, neuroinflammation, neuronal cell death, and cognitive deficits. Wilcock *et al.* have shown that treatment of APPSwDI/NOSC$^{-/-}$ mice with amyloid-beta directed active immunotherapy leads to marked reduction in amyloid deposition, but with increased incidence of microhemorrhages (Wilcock *et al.*, *J Neurosci.* 29:7957–7965, 2009).

[0168] In an *in vivo* AD model, the progression of AD is investigated by examining immunohistochemical signatures of amyloid deposition, tau hyperphosphorylation, and BBB leakage (fibrinogen), as well as by assessing cognitive abilities in spatial memory-based behavioral paradigms. In this model, the transgenic mice are administered MAb 67

or Control Ig (Mab 2B8) intravenously at a concentration of 30mg/kg from age 26 to 38 weeks for a total of 13 doses.

[0169] The mice are initially subjected to baseline behavioral testing at age 10-12 weeks, *e.g.*, Open field, RAWn and Barnes Maze tests, and mice reaching the criteria of activity and learning/memory are included in the follow-up. Behavioral deficits are again measured at age 38, 39 and 40 weeks and body weight is recorded. Mice that do not reach criteria for study enrollment will be sacrificed. At the 41 week of age end-point, the animals will be euthanized and the brains will be processed for biochemical and immunohistological analyses for soluble and insoluble amyloid beta levels and deposits. Serum is collected pre-dosing, during dosing and at the end-point for PK at age 10, 25 and 41 weeks. Tissue sections from the central nervous system (CNS) of animals at different stages of disease may be immunostained for fibrinogen, that can be used as markers for BBB disruption.

[0170] Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims and list of embodiments disclosed herein. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

WHAT IS CLAIMED IS:

1. A method of decreasing blood brain barrier permeability in a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D), thereby decreasing blood brain barrier permeability in the subject.

2. A method of maintaining or increasing Claudin-5 expression in a subject having a neuroinflammatory disorder comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D), wherein the binding molecule maintains or increases Claudin-5 expression in the subject.

3. The method of claim 1 or 2, wherein the binding molecule inhibits SEMA4D interaction with Plexin-B1.

4. A method of decreasing blood brain barrier permeability in a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically inhibits semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor, thereby decreasing blood brain barrier permeability in the subject.

5. A method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically inhibits semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor, wherein the binding molecule decreases permeability of the blood-brain barrier, thereby treating the subject.

6. The method of claim 4 or 5, wherein the SEMA4D receptor is Plexin-B1.

7. The method of any one of claims 4-6, wherein the binding molecule specifically binds to SEMA4D or Plexin-B1.

8. The method of any one of claims 1-7, wherein the binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D.

9. A method of decreasing blood-brain barrier permeability in a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to SEMA4D, wherein the binding molecule competitively inhibits a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67 from specifically binding to SEMA4D, thereby decreasing blood brain barrier permeability in the subject.

10. The method of any one of claims 1-9, wherein the binding molecule specifically binds to the same SEMA4D epitope as a reference monoclonal antibody selected from the group consisting of VX15/2503 or 67.

11. The method of any one of claims 1-10, wherein the binding molecule comprises an antibody or antigen-binding fragment thereof.

12. The method of claim 11, wherein the antibody or antigen-binding fragment thereof comprises a variable heavy chain (VH) comprising VHCDRs 1-3 comprising SEQ ID NOs 6, 7, and 8, respectively, and a variable light chain (VL) comprising VLCDRs 1-3 comprising SEQ ID NOs 14, 15, and 16, respectively.

13. The method of claim 12, wherein the antibody or antigen-binding fragment thereof is monoclonal antibody VX15/2503 or 67.

14. The method of claim 7, wherein the binding molecule specifically binds to Plexin-B1.

15. The method of claim 14, wherein the binding molecule competitively inhibits SEMA4D from binding to Plexin-B1.

16. The method of claims 14 or 15, wherein the binding molecule is an anti-Plexin-B1 antibody or antigen binding fragment thereof.

17. A method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an isolated binding molecule which specifically binds to semaphorin-4D (SEMA4D) and an isolated binding molecule which specifically binds to Plexin-B1, wherein the SEMA4D and Plexin-B1 binding molecules decrease permeability of the blood-brain barrier, thereby treating the subject.

18. A method of treating a subject having a neuroinflammatory disorder, comprising administering to the subject an effective amount of an inhibitor of semaphorin 4D (SEMA4D) interaction with a SEMA4D receptor, wherein the inhibitor decreases permeability of the blood-brain barrier, thereby treating the subject.

19. The method of claim 18, wherein the inhibitor is selected from the group consisting of an anti-SEMA4D binding molecule, an anti-PlexinB1 binding molecule, a small molecule inhibitor of SEMA4D, or a small molecule inhibitor of a SEMA4D receptor.

20. The method of any one of claims 1-19, wherein the neuroinflammatory disorder is selected from the group consisting of Multiple Sclerosis, Amyotrophic Lateral Sclerosis, epilepsy, Alzheimer's Disease, Parkinson's Disease, meningitis, brain edema, and brain trauma.