(54) Title: USE OF AN MMP28 INHIBITOR FOR INCREASING MYELINATION

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USE OF AN MMP28 INHIBITOR FOR INCREASING MYELINATION

FIELD OF THE INVENTION

The present invention relates to the use of an MMP28 inhibitor for increasing myelination of the peripheral nervous system and the central nervous system. The methods of the present invention are accomplished by administering an MMP28 inhibitor, e.g., an anti-MMP28 antibody, to a subject with a demyelination or insufficient myelination disorder, disease or condition.

BACKGROUND OF THE INVENTION

Myelin, a substance rich in lipid and protein, surrounds the axons of many neurons in both the central nervous system (CNS) and peripheral nervous system (PNS) of vertebrates, thereby forming the myelin sheath. Myelin acts as insulation around the axons and functions to promote efficient transmission of a nerve impulse along the axon.

Demyelination is the net degradation or destruction of the myelin sheath surrounding axons resulting in disruption of signals. CNS demyelination is a hallmark of certain neurodegenerative diseases including multiple sclerosis, epilepsy, transverse myelitis, chronic inflammatory demyelinating polyneuropathy and adrenoleukodystrophy (ALD). CNS demyelination also may occur as a result of exposure to certain toxins (e.g., heavy metals, metal chelators, taxol, suramin and lyssolecithin), or infection with certain viruses (e.g., HIV and herpes virus) or mycobacterium (Mycobacterium leprae). Certain mental disorders such as autism, attention deficit/hyperactivity disorder, schizophrenia, bipolar disorder, depression and Alzheimer’s disease are associated with insufficient myelination or demyelination in the CNS.

PNS demyelination is associated with a number of diseases, disorders or conditions including diabetic neuropathy, Guillain-Barre disease (acute demyelinating polyneuropathy), chronic inflammatory demyelinating polyradiculoneuropathy (CIPD), and HIV inflammatory demyelinating disease. Axon damage due to physical trauma may also result in demyelination in both the PNS and CNS.

Although demyelination, or insufficient myelination, diseases, disorders or conditions affect millions of people worldwide, treatments are limited. For example,
while several types of therapy, predominantly immune system suppressants, have proven to be helpful for treatment of multiple sclerosis, they are limited in number, efficacy and, in some instances, by toxic effects. Certain demyelination diseases, e.g., ALD, have been treated with limited success by modifying the diet; however, this approach is best if used prior to the onset of disease symptoms. For certain types of demyelination diseases, e.g., leukodystrophies, progression may be slowed by bone marrow transplantation but the diseases are primarily treated symptomatically.

Matrix metalloproteinases (MMPs) comprise a family of endopeptidases capable of degrading extracellular matrix (ECM) components as well as several cell surface and pericellular proteins. MMPs also regulate many processes during development and in adulthood, particularly those involving ECM remodeling. Although MMPs share several common structural features and generally have ECM-related functions, they are functionally distinct. MMP28, also referred to as epilysin, is the newest member of the matrix metalloproteinase (MMP) family. PCT International Publication No. WO02/20739 discloses nucleic acid molecules which encode two alternative forms of MMP28. The function of MMP28 is not well understood; however increased MMP28 in certain cell types has been associated with multiple disease states including wound repair, osteoarthritis and some types of cancer.

There is a great need for alternative therapies for diseases, disorders or conditions characterized by demyelination or insufficient myelination in the CNS and/or the PNS. There are currently limited therapies which promote myelination. The present application describes the unexpected finding that MMP28 inhibitors, particularly anti-MMP28 antibodies, may be used to increase myelination and therefore to treat many recalcitrant nervous system diseases, disorders or conditions.

**SUMMARY OF THE INVENTION**

The present invention is directed to the discovery that MMP28 degrades myelin and that inhibitors of MMP28 increase myelination of the CNS and PNS. Such inhibitors include e.g., an anti-MMP28 antibody, small molecule, peptide, ribozyme or antisense molecule.

Accordingly, the present invention provides use of an MMP28 inhibitor, e.g., an anti-MMP28 antibody, small molecule, peptide, ribozyme or antisense molecule, in the
manufacture of a medicament for increasing myelination in a human subject. In one embodiment, the invention provides use of an anti-MMP28 antibody in the manufacture of a medicament for increasing myelination in a human subject.

The invention embodies the use of an MMP28 inhibitor, preferably an anti-MMP28 antibody, in the manufacture of a medicament for the treatment of diabetic neuropathy, acute demyelinating polyneuropathy, chronic inflammatory demyelinating polyradiculoneuropathy, HIV inflammatory demyelinating disease, herpes virus infection, Hansen’s disease, axon damage due to physical trauma or increased pain sensitivity with axon damage.

The invention further embodies the use of an MMP28 inhibitor, preferably an anti-MMP28 antibody, in the manufacture of a medicament for the treatment of multiple sclerosis, leukodystrophy, Charcot-Marie-Tooth disease or spinal cord injury.

The invention also embodies the use of an MMP28 inhibitor, preferably an anti-MMP28 antibody, in the manufacture of a medicament for the treatment of Alzheimer’s disease, schizophrenia, major depressive disorder, bipolar disorder or attention deficit hyperactivity disorder.

One embodiment of the invention provides a method for increasing myelination, in a human subject suffering from a disease, disorder or condition which benefits from an increase in myelination comprising administering to the human subject an effective amount of an MMP28 inhibitor, preferably an anti-MMP28 antibody.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1A shows the amino acid sequence of the full-length human pro-MMP28 protein (SEQ ID NO:1) with the sequence underlined which is removed to generate active MMP28. The metalloprotease active site is in bold print and extends from amino acids 240-250. The furin cleavage site extends from amino acids 118-122.

FIG. 1B shows the amino acid sequence of the active form of human MMP28 (SEQ ID NO: 7)

FIG. 2 shows an alignment of the amino acid sequence of the full-length pro-MMP28 protein of Xenopus (X)(SEQ ID NO: 4), Human (H) (SEQ ID NO: 1), Murine
(M)(SEQ ID NO: 5), Rat (R)(SEQ ID NO: 6), and the amino acids identical to X, H, M and R (C).

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based on the finding that MMP28 protein degrades certain myelin-associated proteins and that inhibitors of MMP28, particularly anti-MMP28 antibodies, increase myelination in the CNS and PNS.

"MMP28," unless otherwise indicated, refers to the active form of MMP28 protein. The active form of human MMP28 protein, generated by furin cleavage of the full-length form, has the amino acid sequence shown in SEQ ID NO: 7. The full-length form of human MMP28 has the amino acid sequence shown in SEQ ID NO: 1. The propeptide region – underlined in Fig. 1A – is removed upon furin-cleavage to generate the active form of the protein.

The term "subject," as used herein, refers to a mammal, preferably a human. In a certain embodiment, the subject is further characterized with a disease, disorder or condition that would benefit from an increase in myelination in the CNS or PNS.

"Demyelination" is the net degradation or destruction of previously existing myelin.

"Insufficient myelination" is the lack of sufficient myelin surrounding an axon to perform the normal activity of myelin.

A medicament which has the effect of "increasing myelination" increases the net amount of myelin associated with the axon subsequent to administration of the medicament to the subject. The amount of myelin associated with the axon may be measured by a method known in the art such as magnetic resonance imaging, particularly diffusion tensor imaging.

The term “antibody,” in reference to an anti-MMP28 antibody, as used herein, refers to a monoclonal or polyclonal antibody that binds MMP28, preferably human MMP28. A “monoclonal antibody” as used herein refers to a chimeric antibody, a humanized antibody or a human antibody. “Monoclonal antibody” refers to an antibody that is derived from a single copy or clone, including e.g., any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.
“Antibody” as used herein, can be (i) an intact antibody (comprising two full-length light chains and two full-length heavy chains), (ii) a fragment of an antibody comprising an antigen-binding portion, e.g., a Fab, Fab’, or F(ab’)2, or (iii) a single chain Fv fragment that may be produced by joining the DNA encoding the light chain variable region (LCVR) and heavy chain variable region (HCVR) with a linker sequence. (See, Pluckthun, The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp 269-315, 1994). It is understood that regardless of whether antigen-binding fragments are specified, the term “antibody” as used herein includes such fragments as well as single chain forms, unless indicated otherwise. As long as the antibody protein retains the ability to bind MMP28, it is included within the term “antibody” or “anti-MMP28 antibody” as used herein.

The term “humanized antibody” as used herein refers to an antibody wherein at least one portion is of human origin. For example, a humanized antibody can comprise portions derived from an antibody of nonhuman origin, such as a mouse, and portions derived from an antibody of human origin.

There are multiple methods available in the art to generate humanized antibodies. For example, humanized antibodies may be produced by obtaining nucleic acid sequences encoding the HCVR and LCVR of a parent antibody (e.g., a murine antibody or antibody made by a hybridoma) which binds MMP28, identifying the CDRs in said HCVR and LCVR (nonhuman), and grafting such CDR-encoding nucleic acid sequences onto selected human framework-encoding nucleic acid sequences. Optionally, a CDR region may be optimized by mutagenizing randomly or at particular locations in order to substitute one or more amino acids in the CDR with a different amino acid prior to grafting the CDR region into the framework region. Alternatively, a CDR region may be optimized subsequent to insertion into the human framework region using methods available to one of skill in the art.


A human antibody is an antibody obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge.
One method for generating fully human antibodies is through the use of XENOMOUSE™ strains of mice that have been engineered to contain human heavy chain and light chain genes within their genome (see, e.g., Mendez et al., Nature Genetics 15:146-157, 1997). XENOMOUSE™ strains are available from Abgenix, Inc.

The present invention provides the use of an anti-MMP28 antibody for the manufacture of a medicament for increasing myelination in a subject. Preferably the subject is a human.

In a preferred embodiment, the anti-MMP28 antibody is a monoclonal antibody, more preferably a human or humanized monoclonal antibody. In another preferred embodiment, the anti-MMP28 antibody binds a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 3.

In one embodiment, an MMP28 inhibitor, preferably an anti-MMP28 antibody, can be administered to a subject, to increase myelination of the CNS. Preferably the subject is a human subject that has a disease, disorder or condition characterized by demyelination or insufficient myelination of the CNS. Such diseases, disorders or conditions of the CNS include, but are not limited to, multiple sclerosis, epilepsy, leukodystrophy, Charcot-Marie-Tooth disease and spinal cord injury. Leukodystrophies are mostly inherited disorders and include adrenoleukodystrophy, metachromatic leukodystrophy, Krabbe disease, Pelizaeus-Merzbacher disease, childhood ataxia with central hypomyelination, Canavan disease, Alexander disease, Refsum disease and cerebrotendinous xanthomatosis. Additionally, there is increasing evidence that certain mental conditions or disorders are characterized by insufficient myelination and/or demyelination, e.g., Alzheimer’s disease, schizophrenia, major depressive disorder, bipolar disorder and attention deficit hyperactivity disorder. The use of an anti-MMP28 antibody in the manufacture of a medicament for increasing CNS myelination in a subject, preferably a human subject that has at least one of the aforementioned diseases, disorders or conditions is contemplated.

In one embodiment, an MMP28 inhibitor, preferably an anti-MMP28 antibody, can be administered to a subject to increase myelination of the PNS. Preferably, the subject is a human subject that has a disease, disorder or condition characterized by demyelination or insufficient myelination of the PNS. Such diseases, disorders or conditions include diabetic neuropathy, Guillain-Barre disease (also referred to as acute
demyelinating polyneuropathy), chronic inflammatory demyelinating polyradiculoneuropathy (CIPD), HIV inflammatory demyelinating disease, herpes virus infection, axon damage due to physical trauma (e.g. Wallerian degeneration), Hansen’s disease, and increased pain sensitivity with axon damage. The use of an anti-MMP28 antibody in the manufacture of a medicament for increasing PNS myelination in a subject, preferably a human subject that has at least one of the aforementioned diseases, disorders or conditions is contemplated.

In a preferred embodiment of the present invention, a pharmaceutical composition comprising an anti-MMP28 antibody is administered in an effective amount to a subject for increasing myelination. Preferably the pharmaceutical composition comprises a homogeneous or substantially homogeneous population of an anti-MMP28 antibody and a pharmaceutically acceptable carrier or diluent. The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, generally inert vehicle for the active agent, which does not adversely affect the agent or the subject to whom the composition is administered. Suitable vehicles or carriers can be found in standard pharmaceutical texts, for example, in Remington’s Pharmaceutical Sciences, 16th ed. Mack Publishing Co., Easton, PA (1980). Such carriers include, e.g., aqueous solutions such as buffers and physiological saline. In addition, the carrier can contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability or rate of dissolution of the formulation.

The pharmaceutical composition must be sterile and stable under the conditions of manufacture and storage in the container provided, including e.g., a sealed vial, syringe or other delivery device, e.g., a pen. Therefore, pharmaceutical compositions may be sterile filtered after making the formulation, or otherwise made microbiologically acceptable.

The manner of administering a pharmaceutical formulation containing an anti-MMP28 antibody can be intravenous, subcutaneous, intracranial, intrathecal, intracranial or intramuscular. Certain diseases such as multiple sclerosis may have a breakdown of the blood-brain barrier which facilitates delivery of the antibody by intravenous route. For other diseases, disorders or conditions characterized with an intact blood-brain barrier, a preferred means of administering the pharmaceutical formulation is directly into the brain via intracranial ventricular infusion with the aid of catheters and pumps.
An “effective amount” refers to an amount necessary (at dosages and for periods of time and for the means of administration) to increase myelination in the CNS and/or PNS of the subject to whom it is administered. An effective amount of an anti-MMP28 antibody may vary according to factors such as the disease state, age, sex, weight of the individual, means of administration and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the antibody, are outweighed by the therapeutically beneficial effects.

An effective amount is at least the minimal dose, but less than a toxic dose that is necessary to impart therapeutic benefit to a subject. Stated another way, an effective amount of an anti-MMP28 antibody is an amount which, preferably in humans, (i) increases myelination in the PNS, and/or (ii) increases myelination in the CNS.

As is well known in the medical arts, dosages for any one subject depends upon many factors, including the patient’s size, body surface area, age, the particular compound to be administered, sex, time and route of administration, frequency of administration, general health, and other drugs being administered concurrently. Dose may further vary depending on the type and severity of the disease. A typical dose can be, for example, in the range of 0.001 to 1000 mg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. A weekly parenteral dosage regimen can be about 0.1 µg/kg to about 20 mg/kg of total body weight, preferably from about 0.3 µg/kg to about 10 mg/kg. Progress may be monitored by periodic assessment, and the dose adjusted accordingly.

These suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained. Factors for consideration in this context include the particular disorder being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

30 MMP28 Expressed in CNS and PNS

The spatio-temporal localization of MMP28 was evaluated in developing mouse embryos. Sections of whole embryos were fixed daily from embryonic day 10 (E10) to
E17 and stained for MMP28 protein. Using standard immunohistochemistry techniques, MMP28 protein levels and localization were found to vary depending on the developmental stage. The anti-MMP28 antibody used was CL2MMP-28 of Cedarlane Labs. Secondary antibody and peroxidase labeling was carried out according to Vectastain ABC kit (Vector labs).

At E10 to E12, MMP28 expression was low throughout the embryo. Beginning at E13, MMP28 expression increased, with staining strongest in the developing spinal cord. At E13, MMP28 expression was not yet found in the nerves of the extremities. At E14, expression of MMP28 increased dramatically throughout the nervous system. During later stages of development (E15-E17), neural-associated MMP28 was reduced but remained detectable throughout the CNS. Neural MMP28 expression was present in both the CNS and PNS. Nerves within the limbs were found to express MMP28 at E14 as were nerves through the body. Within the CNS, MMP28 was expressed strongly in the brain and spinal cord. Cranial nerves were also found to express MMP28. MMP28 was also found to be expressed in the spinal cord or 12 week old mice as detected by immunofluorescence and confocal microscopy.

MMP28 Degrades Certain Myelin Associated Proteins

Polypeptides resulting from MMP28 cleavage activity in the neural extracellular environment were identified. Given the conserved expression pattern between Xenopus MMP28 (XMMMP28) and mammalian MMP28 and the similarity at the amino acid sequence level (see Fig. 2), it is expected that Xenopus MMP28 and mammalian MMP28 cleave the same or similar substrates. Full length XMMMP28 is 56% identical to full-length human MMP28 and 55% identical to full-length murine MMP28. Like its mammalian counterpart, XMMMP28 consists of a signal sequence and pro-domain containing an inhibitory cystein switch, followed by the catalytic domain and C-terminal hemopexin-like domain. Homology across Xenopus, murine and human MMP28 is highest within the putative functional areas: catalytic domain (65% identity), inhibitory cysteine switch (85% identity) and furin cleavage signal (100% identity).

Xenopus MMP28 (XMMMP28, SEQ ID NO: 4) was incubated in protease buffer (50 mM HEPES, pH 7.0, 10 mM CaCl2, 0.05% Brij-35, and 10 μM ZnCl2) with tissue from E17 brains of Long-Evans rats at 37°C for 24 hours. Brain digests were then
centrifuged through a 3 kDa filter to remove debris and larger proteins. The smaller peptides which pass into the flow-through were treated with trypsin to reduce the size of the peptides and subjected to separation of peptides by liquid chromatography followed by peptide mass spectroscopy to identify the peptides generated after XMMP28 degradation. Peptides corresponding to myelin components Nogo-A, predominantly associated with CNS expression, (RGSGSVDETFLPAASEPVIPSSAEKI, SEQ ID NO: 9) and neural cell adhesion molecule, i.e., NCAM-1, expressed in both CNS and PNS, (KSEPKQSEAKPAPTEVK, SEQ ID NO: 8) degradation products were specifically identified in XMMP28-treated samples, but were missing or found at lower relative abundance in no-enzyme control samples.

Western blot analysis of the myelin-associated proteins Nogo-A, NCAM-1, myelin basic protein, proteolipid protein and myelin associated glycoprotein, i.e., MAG, in these XMMP28/brain digests were performed to determine if XMMP28 incubation results in the degradation of these proteins. Full length Nogo-A protein was reduced in XMMP28 treated brain samples as are NCAM-1 and MAG. Degradation was limited to certain myelin proteins. Myelin basic protein and PLP were not reduced in size in this assay. Therefore, MMP28 cleaves Nogo-A, NCAM-1 and MAG.

Generation of MMP28 Polyclonal Antibodies

Polyclonal antibodies were generated in rabbits to two different peptides (i) FAKQGNKWKYKHSRLY (SEQ ID NO: 2) and (ii) KRLGRDALLSW (SEQ ID NO: 3). These peptides are present within human MMP28, but not present in other known human proteins, including any other human MMP protein. Therefore, antibodies generated to these peptides are not expected to bind any other MMP protein. The peptide with SEQ ID NO: 2 spans amino acids 123-139 of full-length human MMP28 with SEQ ID NO: 1. The peptide with SEQ ID NO: 3 spans amino acids 263-273 of full-length human MMP28 with SEQ ID NO: 1. According to computer-modeled predictions of human MMP28 structure using SWISS-Model, these two peptides exist on the surface of the human MMP28 protein and lie in opposition to the metalloprotease active site.

Each of the peptides was synthesized, conjugated to KLH and injected into rabbits using a standard immunization scheme. The resulting MMP28 antibodies in the serum of the immunized rabbits were affinity purified against the peptide to which they were
generated using standard affinity chromatography techniques. A second round of
antibody purification was performed using Protein G sepharose in Ab Spin Trap columns
(GE Healthcare Lifesciences). The antibodies were eluted in 800 µl of 0.1 mM glycine,
pH 2.5 and neutralized with 60 µl 1 M Tris-HCl, pH 9.0. Concentration of the purified
antibodies was determined by spectrophotometer reading at 260 nM.

The rabbit-generated antibodies, AB183 and AB180, specifically bind purified
human MMP28 but do not bind human MMP2 as was demonstrated by Western blot
analysis. AB183 was generated against and binds the polypeptide with the sequence
shown in SEQ ID NO: 2, both independently and within the context of human MMP28
protein. AB180 was generated against and binds the polypeptide with the sequence
shown in SEQ ID NO: 3, both independently and within the context of human MMP28
protein.

The following examples are offered for illustrative purposes only, and are not
intended to limit the scope of the present invention.

Example 1 AB183 and AB180 inhibit MMP28 activity in vitro

This assay determines the activity of purified human MMP28 protein (a mixture
of full-length and active form) on a peptide substrate and the ability of anti-MMP28
antibodies, AB183 and AB180, to modulate the MMP28 activity. Increase in
fluorescence is measured after incubation of a fluorogenic pan-MMP substrate with
purified MMP28. Human MMP2 is used as a positive control. OMNI-MMP™, a highly
quenched fluorogenic peptide substrate for most MMPs (Biomol, Plymouth Meeting,
PA), is used for these assays after dilution in DMSO to 20 mM.

The MMP protein to be tested (i.e., MMP28 or MMP2), with or without double-
purified AB180 or AB183, is combined with water in a final 50 µl volume such that the
MMP protein is 20 nM and the antibody is either 60 nM or 10 nM. The MMP/AB
mixture is incubated at 37°C for one hour with constant shaking. The fluorogenic peptide
substrate is then added to the MMP/AB mixture to a final concentration of 10 µM along
with a 10X protease assay buffer such that the final 100 µl reaction is in 50 mM HEPES,
pH 7.0, 10 mM CaCl₂, 0.05% Brij-35, and 10 µM ZnCl₂. The reactions are carried out in
black 96-well plates covered with aluminum foil and incubated at 37°C for 24 hours.
Fluorescence is then measured at 340 nM excitation, 405 nM emission for 1 second/well.
MMP28 alone, when examined between 0 nM and 80 nM, cleaves the substrate in a dose dependent manner. A similar result is obtained with MMP2 alone. AB180 at a final concentration of 5 nM (in the 100 µl reaction) inhibits MMP28 cleavage of the substrate about 66%, while a final concentration of 30 nM AB180 inhibits MMP28 cleavage of the substrate about 98% as reflected by diminished fluorescence. AB183 at a final concentration of 5 nM inhibits MMP28 cleavage of the substrate about 60%, while a final concentration of 30 nM AB183 inhibits MMP28 cleavage of the substrate about 69%. Both antibodies significantly decreased substrate cleavage (p<0.05) compared to 10 nM MMP28 alone. No detectable inhibition of cleavage of the substrate is observed when AB180 or AB183 are used with MMP2.

**Example 2** MMP28 inhibitors, AB180 and AB183, increase myelination

Myelinating dorsal root ganglion (DRG) cultures are established as described in Svenningsen, A., et al. *J. Neurosci Res.* 72:565-573, 2003. Embryos are isolated at day 17 of gestation from pregnant Long-Evans rats and placed in cold L15 medium. Dorsal root ganglia are trimmed away from the spine and placed in 2 ml L15 medium in a 60 mm dish and treated with 0.25% trypsin at 37°C for 15 min. The cells are dissociated using a pipette tip and dissociation is verified by microscopic examination. The cells are then washed in L15 medium with 10% fetal bovine serum 3 times and resuspended in Neuralbasal media (Invitrogen, 21103) with 100 ng/ml nerve growth factor (NGF) and 2% B27 supplement (Growth Media). The cells are grown (37°C in 5% CO₂) for 4 days, fresh Growth Media containing 50 µg/ml ascorbic acid is then added to initiate myelination and replaced with same every 2-3 days.

In these experiments, axon-associated Myelin Associated Glycoprotein (MAG) is used as a biomarker for early myelination (Owens and Bunge, *Glia* 2:119-128, 1989). Neural expression of MMP28 and MAG is monitored using standard immunohistochemistry techniques with an anti-MAG antibody (Chemicon) or anti-MMP28 antibody (Cederlane). Prior to initiation of myelination and at days 1 and 3 after initiation of myelination, there is no detectable MAG in the DRG cultures, neither the glial cells (i.e., the myelin-producing Schwann cells) nor the axons. However, there is a baseline level of MMP28 observable in the axons. MMP28 is never observed in the glial cells. Six days after initiation of myelination, initial stages of myelination are detectable
by the presence of MAG within the glial cells particularly along some positions of neural-glial cell interaction. At this stage of growth, MMP28 levels are not notably decreased. Fourteen days after initiation of myelination, there is axonal association of MAG and substantially reduced levels of MMP28. Therefore, when myelination is not proceeding, MMP28 levels are at baseline and MAG levels are undetectable; however, when myelination is proceeding (i.e., increasing myelination), MMP28 levels decline and MAG levels increase.

Addition of AB180 or AB183 to the cultures on day 6 to a concentration of 30 nM results in an increase in axon-associated MAG, observable by immunohistochemistry staining, after 24 hours. The effect of AB180 and AB183 is quantifiable by counting the number of axon bundles which are positive for MAG staining in the treated and untreated wells. On day 7, the axon bundle numbers in a total of six wells, with and without antibody treatment, are summed. The resulting figures are presented in Table 1 below. These data demonstrate that MMP28 antibodies significantly (p≤0.05) enhance the formation of axon-associated myelin. This assay may be used to test any potential MMP28 inhibitor.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Total Axons</th>
<th>MAG Positive Axons</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Antibody</td>
<td>152</td>
<td>5 (3.3%)</td>
</tr>
<tr>
<td>AB180, 5nM</td>
<td>155</td>
<td>12 (7.7%)</td>
</tr>
<tr>
<td>AB183, 5nM</td>
<td>165</td>
<td>24 (14.5%)</td>
</tr>
</tbody>
</table>

**Example 3** In vivo cuprizone assay

Mice are fed 0.2% cuprizone in the diet for 3-13 weeks. This toxin results in the demyelination of heavily myelinated regions of the brain, including the corpus callosum, and spinal cord (Matsushima GK, et al., *Brain Pathol* 2001;11:107Y16). Detection of demyelination may be conducted by MRI or histology of the corpus callosum. When cuprizone is removed from the diet, remyelination occurs with greater myelination capacity in mice treated for shorter periods of time. Enhancement of myelination is assessed during this remyelination period by radiologic evaluation as well as histology. Antibody treatment is through intraperitoneal injection. Antibodies that specifically bind MMP28 are expected to enhance remyelination or promote myelination.
Example 4  MMP28 Expression in MS lesions

A. Human Brain Lesions

This example demonstrates that MMP28 protein levels are elevated in human brain lesions from a human patient with Multiple Sclerosis (MS) as compared to normal brain tissue. Frozen 5μm cerebellar tissue sections from a human MS patient (Biomax, Ijamsville, MD) are thawed to room temperature and placed in 0.1% Luxol fast blue solution (0.1g Luxol fast blue (Acros), 0.5ml acetic acid, in 95% ethanol to 100 ml) for 16 hours at 56°C. The slides are then removed from Luxol fast blue, washed in 95% ethanol, rinsed in distilled, deionized water (ddH₂O) and differentiated in 0.05% lithium carbonate (Acros) for 30 seconds. Following differentiation, slides are then rinsed in ddH₂O and examined microscopically to verify differentiation of white matter. The slides are then incubated in 0.1% Cresyl echt violet (American Master Tech Scientific) for 40 seconds to counterstain nuclei and gray matter. Excess Cresyl echt violet is rinsed off the slides with ddH₂O. The slides are differentiated in 95% ethanol for 5 minutes followed by sequential dehydration in 100% ethanol and Xylenes. The sections are permanently mounted under coverslips using Permount. The tissue is analyzed by light microscopy using an upright microscope. To identify changes in protein expression within MS lesions, immunohistochemistry is performed using standard techniques with antibodies to MMP-28 (Cerderlane) and MAG (Chemicon). Nuclei are counterstained with DAPI (4’,6-diamidino-2-phenylindole, Sigma).

Performing the staining described above, lesions with reduced myelination are identified in cerebellar lesions from a patient with multiple sclerosis by Luxol blue staining. These regions are in areas of the cerebellum expected to be myelinated and surrounded by normal, myelinated tissue evidenced with more intense Luxol blue staining. To confirm reduced myelin, immunohistochemistry is performed on the next serial section for the myelin protein Myelin Associated Glycoprotein (MAG). As expected, in regions identified as normal by Luxol blue staining, MAG protein is strongly expressed indicating normal myelination. Within the boundaries of the identified MS lesions, MAG staining is substantially reduced. Immunohistochemistry to MMP-28 is performed on the same section. Throughout the normal regions of the cerebellum, no axon associated MMP-28 staining is identified, however throughout the MS lesions,
numerous structures presumed to be axons based on morphology stained positive for MMP-28. MAG staining was not found to co-localize to these structures. These results demonstrate that in normal, myelinated cerebellum, MMP-28 expression is not expressed at significant levels while in regions of demyelination, there are demyelinated axons in which MMP-28 is expressed.

B. EAE Model Spinal Cord Lesions

EAE is a CDR+ T cell-mediated demyelinating disease of the central nervous system that serves as a model for MS in humans. A recent review discussing spinal cord lesions in human MS patients notes that such lesions correlate well with disease symptoms (Edwards, et al. Expert Review of Neurotherapeutics 7:1203-1211, 2007). For disease induction, approximately 8 week old female C57BL/6 mice are subcutaneously immunized in the right flank on day 0 and day 7 with 200 μL of Complete Freund’s Adjuvant (CFA) containing 2.5 mg/ml H37 RA powder (5 mg/ml, Difco #231141) and 1.5 mg/ml MOG35-55 (myelin oligodendrocyte glycoprotein; Peptide International, PMG-3660-P1) emulsified in CFA with H37 RA/PBS (1:1) by sonication on ice. Pertussis toxin (200 μL of 2.5 μg/ml) is administered at day 0 with the first immunization and again at 48 hours after the first immunization.

The pathology of disease is allowed to progress and the mice are sacrificed 4 weeks after immunization at which time the animal is demonstrating reduced motor control. The spinal cord is isolated at the time of sacrifice and longitudinal spinal cord sections are stained using standard immunohistochemistry techniques with an anti-MAG antibody (Chemicon) and an anti-MMP28 antibody (Cederlane). Axons within the spinal cord of EAE mice express MMP28 and this MMP28 expression is within the axons that have thin or no MAG surrounding them. No MMP-28 positive axons are identified in the spinal cord of normal mice.
WE CLAIM:

1. Use of an MMP28 inhibitor in the manufacture of a medicament for increasing myelination in a human subject.
2. Use of an anti-MMP28 antibody in the manufacture of a medicament for increasing myelination in a human subject.
3. Use according to claim 1 or 2, wherein the myelination increase occurs within the peripheral nervous system.
4. Use according to claim 1 or 2, wherein the myelination increase occurs within the central nervous system.
5. Use of an MMP28 inhibitor in the manufacture of a medicament for the treatment of diabetic neuropathy, acute demyelinating polyneuropathy, chronic inflammatory demyelinating polyradiculoneuropathy, HIV inflammatory demyelinating disease, herpes virus infection, Hansen’s disease, axon damage due to physical trauma or increased pain sensitivity with axon damage.
7. Use of an MMP28 inhibitor in the manufacture of a medicament for the treatment of Alzheimer’s disease, schizophrenia, major depressive disorder, bipolar disorder or attention deficit hyperactivity disorder.
8. Use according to any one of claims 5 to 7, wherein the inhibitor is an anti-MMP28 antibody.
9. Use according to any one of claims 2, 3, 4 or 8, wherein the antibody is a monoclonal antibody.
10. Use according to claim 9, wherein the antibody is human.
11. Use according to claim 9, wherein the antibody is humanized.
12. Use according to claim 9, wherein the antibody is chimeric.
13. Use according to any one of claims 2 to 4 and 8 to 12, wherein the antibody binds a polypeptide consisting of the amino acid sequence shown in SEQ ID NO: 2 or SEQ ID NO: 3.
FIG. 1

A. Human MMP28 Full-Length Sequence (SEQ ID NO: 1)

MVARVGLLRL ALQLLLWGLH DAQPAERGGQ ELRKEAEAFL EKYGYLNEQV 50
PKAPTSTRFS DAIرافQWVS QLPVGSVLDR ATLROMTRPR CGVTDTNSYA 100
AWAERISDLF ARHTKMRRK KRFAKQGNKW YKQHLSYRLV NWPEHLPFEPA 150
VRGAVRAAFQ LWSNVSALEF WEAPATGPAD IRLTFFQGDH NDGLGNADFQG 200
PGGALAHAFPL PRREGAHFQ DERRWSLSRRR GRNL芙VLAH EIGHTGLTH 250
SPAPRALMAP YYKRLGRDAL LSWDDVLAVQ SLYGKPLGGS VAVQLPGKLF 300
TDFETWDYS PSQGRPETQG PKYCHSSFDA ITVDRQQQLY IFKGSFWEVEV 350
AADGNVSEPR PLQERWVGFLP NIEAAAVSL NGDFYFFKG CRCWFRGRPK 400
PVWGLFLQR CR AGGLPRHPDA ALFFPPLRRL ILFKGARYYYV LARGGLQVEP 450
YYPRSLQDQG GIPEEVSGAL PRPDGSIIFRD RDDRYWCLDQ AKLQATTSGR 500
WATELPWMC WHANSGSALF 520

B. Human MMP28 Active Form (SEQ ID NO: 7)

FAKQGNKWKLYKQLSYRLVNWPEHLPFEPAVRGAVRAAFQLWSNVSALEFWEAPATGPADIR
LTFFEQGDHNDGLGNADFQPGGALAHAFPLPRREGAHFQDERRWSLSRRRGRNL芙VLAHEI
GHTLGLTHSPARALMAPYYKRLGRDALLSWDDVLAVQSLYGKPLGGSVAVQLPGKLFDTD
FETWDYSPOPQGRPETQGPKYCHSSFDAITVDRQQQLYIFKGSFWEVEVAADGNVSEPRL
QERWVGLPNNIEAAAVSLNDGFYFFKGRCWFRGRPKPVWGLPQLCRAGGLPRHPDAAL
FFPPLRLRLILFKGARYYYVLARGGLQVEPYYPRSLQDQGIGPEEVSGALPRPDGSIIFRFD
DRYWCLDQAKLQATTSGRWWATELPWMCWHANSGSALF
FIG. 2 - MMP28 Alignment

<----------Prodomain---------->
X -----MEADIP SLFLLLVTIG LCLCNGYISE ETLQTAQVFL EKYGYLTEE-
H MVARVGLLLR ALQLLLLNGHDL DAQPAERRGQ ELRKEAEAFNL EKYGYLNEQV
M MVAGVSSLRL ALPLLLNWGCQ DAQPTQHGLP ELRQEAEAFNL EKYGYLSEQG
R MVTGLSLLLR VPQPLLWGCQ DAQPTPRGHP EMLHQEAEAFNL EKYGYLSEQG
C L LLL E FL EKYGYLYE

<-----Catalytic domain----->
X -----LKLV KSHHHGQRK KRYISKSKKW YKQHLYTYQIV NWPWYLSQHQ
H AWAERISDLF ARHRTYKMRK KRFAKQGNKW YKQHLSYRLV NWPEHLPEPA
M TWTERISTLL AGHRARKMRK KRFAKPGNKW YKQHLYRLIV NWPERLPEPA
R TWTERIRALL AGHRARKMRK KRFAKPGHKW YKQHLSYRLV NWPKSLPEPA
C H R R K KW YKQHLY V NWP L

<-----PA domain----->
X VRQAVKAQFQ LWSNVSSLTF SEALRD-PAD IRLAFFDGDH NDGAGNAFDG
H VRGAVRAAFQ LWSNVSALEF WEAPATGPAD IRLTFFQGDH NDGLGNAFDG
M VRGAVRAAFQ LWSNVSALEF WEAPATGPAD IRLTFFQGDH NDGLANAFDG
R VRGAVRAAFQ LWSNVSALEF WEAPATGPAD IRLTFFQGDH NDGLANAFDG
C VR AV AAFQ LWSNVS L F EA PAD IRL FF GDH NDG NAFDG

<-----Catalytic domain----->
X PGGALAHAF PRGEEAHFDG AEHWNSLNGK- GRNLFEVVLAH EIGHTLGLPH
H PGGALAHAF PRGEEAHFDQ DERWSLLSRRR GRNLFEVVLAH EIGHTLGLTH
M PGGALAHAF PRGEEAHFDG DERWSLLSRRR GRNLFEVVLAH EIGHTLGLTH
R PGGALAHAF PRGEEAHFDQ DERWSLLSRRR GRNLFEVVLAH EIGHTLGLTH
C PGGALAHAF PRGEEAHFD E WSL GRNLFEVVLAH EIGHTLGLH

<-----PA domain----->
X SSFKNALMSP YYKLNKDQV LNFDVLLAIQ NLYGAPP-SG NVQLPGKQF
H SPAPRALMAP YYKKLGNDAL LSDDVLLAVQ SLYGKPLGQS VAQLPGKLF
M SPAPRALMAP YYKKLGNDAL LSDDVLLAVQ SLYGKPLGQS VAQLPGKVF
R SPAPRALMAP YYKKLGNDAL LSDDVLLAVQ NLYGKPLGRS VAQLPGKIF
C S ALM P YYK L D L DDVLA Q YG P QLPKG F
X  AFFQDWSPE- --SHEDSGMK PSYCHISIPDA IWDLKKTLY IFKGRHFWMV
H  TDFETWDSYS PQGRPPETQG PKYCHSSFDA ITVDRQQQLY IFKGSHFWEV
M  TDFEAWDPHN SQSRRPETRG PKYCHSSFDA ITVGDQWRLY VFKGSHFWEV
R  TDFEAWDPHN SQSRRPETRG PKYCHSSFDA ITVGDQWRLY VFKGSHFWEV
C  W  P YCHS FDA IT D  L Y  FK G  F W  V

X  SLGGKISPPQ SLQKRWKKLP SYIEAAVVG S LDGKFYFFKG GRCWRYKDSI
H  AADGNVSEPR PLQERWVGLP FNIEAAAVSL NDGDFYFFKG GRCWRFGRPK
M  TVDGNVSEPR PLQKRWGPLP PGIEAAAVSL EDGDFYFFKG NRCWRFQGTK
R  TADGNVSEPH PLQKRWGPLP SSIEAAAVSL EDGDFYFFKG NRCWRFQGTK
C  G  S  P  LQ RW LP IEAA V  G P FFKG RCWR

X  LEEGFPQKCS MNGLPRRFPDT ALYFQPLGHL VFIFGSKY YY VNEESLTVEP
H  PVWGLPQLCR AGGLPRHPDA ALFFPLPRRL ILFKGARY YV LARGGLQVEP
M  SVWGFQCLCR AGGLPRHPDA ALFFPLPRRL VLFKGSRYYV LAQGGMQVEP
R  SV--FAQCLCR AGGLPRHPDA ALFFPLPRRL VLFKGSRYYV LARGGMQVEP
C  Q  GLPR PD AL F PL L FK G YYV VEP

X  YYPRSRLDHWK GVPANSHSV L THPDGAIYFF KGHQYWIP DQ KKLKVTSBGK
H  YYPRSRLQDWG GPIEEVS GAL FRPDGSIIFF RDDRYWRLDQ AKLQATTSGR
M  YYPRSRLDWA GVPEEVSGAL FRPDGSIIFF RDDHYWHLDQ AKLQRTSSGR
R  YYPRSRLDWA GVPEEVSGAL FRPDGSIIFF RDDHYWHLDQ AKLQRTSSGR
C  YYPRSRL W G P L PDG I FF W DQ KL T SG

X  WAEDLSWIGC KNDVT-----
H  WATELPWMGC WHANSGGALF
M  WATELSWMGC WHANSGGALF
R  WATELSWMGC WHANSGGALFQRLPVTE
C  WA L W GC

X = Xenopus  (SEQ. ID NO: 4)
H = Human  (SEQ. ID NO: 1)
M = Murine  (SEQ. ID NO: 5)
R = Rat  (SEQ. ID NO: 6)
C = Conserved in all