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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING MULTIPLE SCLEROSIS

(57) **Abstract:** Disclosed are polypeptides, compositions and methods for the treatment or prophylaxis of multiple sclerosis. The method involves the steps of administering a polypeptide, or nucleic acid encoding the polypeptide, comprising the GluR2 NTal-3-2 (Y142-K172) amino acid sequence as shown by SEQ ID NO: 1 or SEQ ID NO: 5 to a subject in need of the treatment.

Compositions and Methods for Treating Multiple Sclerosis

FIELD OF THE INVENTION

[0001] The present invention generally relates to polypeptides. The present invention also relates to polypeptides, compositions and methods for treatment or prophylaxis of a neurological condition. More specifically, the present invention relates to polyeptides, compositions and methods for treating multiple sclerosis.

BACKGROUND OF THE INVENTION

[0002] Multiple sclerosis (MS) is a demyelinating disease affecting the central nervous system, in which episodes of inflammation result in a highly variable course and progression of symptoms (Compston, A. & Coles, A., *Lancet* 372, 1502-1517, 2008). Onset is typically between the ages of 30-50, with higher prevalence in women and great geographic variability (Rosati, G., *Neurol Sci 22,* 117-139, 2001). There are a broad range of symptoms, reflecting the diverse anatomical targets of demyelination, but typical syndromes include: weakness, fatigue, loss of vision, cognitive impairment and impaired balance and coordination (Compston, *supra).* MS episodes are erratic in timing as well, leading to the general principle that MS lesions are disseminated in both space (location) and time (Adams, R *et al, Principles of Neurology*. sixth edn, (McGraw-Hill, 1997)). These symptoms can often be sufficient to make the diagnosis of MS, but magnetic resonance imaging (MRI) is also helpful, along with analysis of cerebrospinal fluid and nerve evoked potential measurements. MS typically begins with reversible neurological deficits (relapsing-remitting phase, or RRMS), that progress eventually to fixed disability in later life (secondary progressive phase)(Adams *et al, supra).* Like other aspects of the disease, the pattern, severity and timing of this progression can be very different among patients, with some experiencing profound disability with rapid progression at the outset (primary progressive MS or PPMS), while a small number of other patients have isolated, relatively mild symptoms.

[0003] The cause of MS is not known with certainty, but both genetic and environmental factors can affect susceptibility (Giovannoni, G. & Ebers, G., *Curr Opin Neurol* 20, 261-268, 2007). Prominent candidate genes include HLA DRBl and DQB, which encode part of the human major histocompatibility complex (MHC), apolipoprotein E (APOE) and interferon

gamma (IFN-g)(Giovannoni *et al, supra;* Kantarci, O. & Wingerchuk, D., *Curr Opin Neurol* 19, 248-254, 2006) The most commonly implicated environmental factors are vitamin D/sunlight, and infectious agents (including the Epstein-Barr virus, human endogenous retroviruses or HERV, and human herpesvirus-6 or HHV-6)(Giovannoni *et al, supra).* Although inflammation has been associated with MS, it is unclear whether demyelination is caused by a primary immune process or a neurodegenerative mechanism (Trapp, B. D. & Nave, K. A., *Annu Rev Neurosci* 31, 247-269, 2008). Autoimmune mechanisms in MS have been studied in depth, with CD4+ type 1 helper T-cells having been considered the main effector of the demyelination. Recently, other immune system components have also been implicated, suggesting that a more broad range of leukocytes may be involved, targeting both the myelin sheath and the axons themselves (Hemmer, B., *et al, Nat Rev Neurosci* 3, 291-301, 2002; Smith, T., *et al, Nat Med* 6, 62-66, 2000).

[0004] There is currently no cure for MS, and despite some recent progress in novel treatments, the disease remains a significant therapeutic challenge (Kieseier, B. C., *et al, Curr Opin Neurol* 20, 286-293, 2007). Standard treatment includes corticosteroids aimed at suppressing the inflammatory response during acute relapse, sometimes with plasmapheresis to remove circulating antibodies from the bloodstream (Giovannoni *et al, supra).* Glatiramer acetate and interferon-β-la are also used in RRMS, but are not particularly effective in PPMS, nor in altering the eventual course of MS, even with early intervention (Compston *et al, supra;* Kieseier *et al, supra).* A more specific immunotherapy uses monoclonal antibodies to target particular surface molecules involved in MS. Natalizumab binds to α4 integrin on white blood cells, thereby reducing their numbers, but due to adverse reactions of progressive multifocal leukoencephalopathy (PML), this drug is used only when other treatments have failed (Kieseier *et al, supra).* Other monoclonal antibodies, rituximab (an anti-CD20 antibody) and daclizumab (targeting CD-25), have a similar rationale, but again are not curative, and have other immunological side-effects.

[0005] The most common rodent model of MS consists of injecting myelin oligdendrocyte glycoprotein (MOG) to generate an autoimmune encephalitis (EAE). The EAE model exhibits an increase in mGluR5 expression in combination with decreased mGluRla receptors in cerebellar Purkinje neurons. Treatment of EAE mice with an mGluR5 enhancer

(RO07 11401) improves motor coordination, but mGluR.5 antagonists do not (Fazio, F. *et al. Neuropharmacology* 55, 491-499, 2008). EAE mice also show a transient reduction of Ca^{++}-dependent glutamate release from cerebral cortex synaptosomes, soon after the onset of the clinical signs (Vilcaes, A. A., *et al, J Neurochem* 108, 881-890, 2009). At the same time, the EAE elevates mRNA levels of the glutamate transporters GLT-1 and GLAST in forebrain and cerebellum, in conjunction with the elevation in extracellular glutamate, although protein levels of these transporters are not consistent with the mRNA findings (Mitosek-Szewczyk, K., *et al, Neuroscience* 155, 45-52, 2008). Other animal models of MS, such as the CNTF -/- mouse, have decreased glutamate decarboxylase, among several proteins in the altered in EAE (Linker, R. A. *et al, PLoS One* 4, e7624, 2009). The neurological effects of EAE can also be attenuated by the gap junction blocker carbenoxolone or the glutaminase inhibitor DON (6-diazo-5-oxo-L-norleucine), likely through decreased glutamate release from microglia (Shijie, J. *et al. Tohoku J Exp Med* 217, 87-92 (2009). *In vitro* activation of microglial mGluR2 exacerbates myelin-induced toxicity, while mGluR3 and group III mGluRs activation is protective (Pinteaux-Jones, F. *et al, J Neurochem* 106, 442-454, 2008).

[0006] The role of glutamate in excitotoxicity is well established, but there is evidence that this neurotransmitter system can also modulate immune system function, and may therefore be a novel therapeutic target for inflammatory disorders of the nervous system (Hansen, A. M. & Caspi, R. R. *Nat Med* 16, 856-858, 2010). Glutamatergic excitotoxicity may also be involved in the mechanisms of neuronal damage by inflammation, since activated immune cells release glutamate (Pitt, D., *et al, Nat Med* 6, 67-70, 2000; Groom, A. J., *et al, Ann N Y Acad Sci* 993, 229-275; discussion 287-228, 2003). Decreased expression of glutamate transporters EAAT (excitatory amino acid transporter) 1 and 2 was reported in post-mortem cerebral cortex samples from patients with MS. These changes correlated with the presence of microglial infiltration and demyelination (Vercellino, M. *et al, J Neuropathol Exp Neurol* 66, 732-739, 2007). Glutamate transporters EAAT-1 and -2 are expressed by oligodendrocytes, the myelin-producing cells, and in MS lesions, the expression of these transporters is lost (Pitt, D., *et al, Neurology* 61, 1113-1120, 2003). Both glutamate and AMPA can enhance T-lymphocyte proliferation in response to MOG and MBP (myelin basic protein) exposure, and mGluR3 mRNA and protein are elevated on T-lymphocytes from patients with active MS compared to controls (Sarchielli, P. *et al, J*

Neuroimmunol 188, 146-158, 2007). Antibodies against NMDA receptors have been detected in a case of recurrent optic neuritis with transient cerebral lesions, in both serum and cerebrospinal fluid, further supporting the hypothesis that autoantibodies against glutamate receptors in the CNS may play a role in demyelinating diseases (Ishikawa, N., *et al,* Neuropediatrics 38, 257-260, 2007).

[0007] Excessive glutamate, acting mainly through NMDARs and AMPARs, facilitates Ca^{2+} influx, which can result in excitotoxicity under pathological conditions including ischemia, trauma, hypoglycemia and epileptic seizure (Simon, R.P., *et al, Science* 226, 850-852, 1984; Choi, D.W. *Trends Neurosci.* 18, 58-60, 1995). The inhibition of Ca(2+)-permeable AMPA receptors may be of benefit in MS, based on the observation that mice with *Gria3* mutations that do not express functional GluR3 AMPA receptor subunits, are resistant to demyelination and neurological sequelae in the EAE model (Bannerman, P. *et al, J Neurochem* 102, 1064-1070, 2007). In contrast, mGluR4 knockout mice are more vulnerable to the EAE model, with more T-helper cells becoming the THI_7 type that produce interleukin-17 (Fallarino, F. *et al,* Nat Med 16, 897-902, 2010). Treatment with a selective mGluR4 enhancer appears to be protective against EAE through enhancement of regulatory T_{reg} cells (Fallarino *et al, supra).* The AMPAR subunit GluRl forms a complex with the interferon-gamma (IFN-γ) receptor that, upon activation by IFN-γ, induces cytotoxicity (Mizuno, T. *et al, FASEB J* 22, 1797-1806, 2008). Direct application of AMPA/kainite antagonists NBQX or MPQX reduces the acute and chronic neurological effects of EAE in rats (Smith *et al, supra).* The protective effects of these drugs may not involve immunological mechanisms, since NBQX did not reduce the amount of inflammation or the *in vitro* proliferation of activated T-cells (Pitt *et al, supra).* AMPA-mediated excitotoxity has also been implicated in other neurodegerative disorders, such as ALS (amyotrophic lateral sclerosis), in which motor neurons are primarily affected. Editing of the GluR2 mRNA is altered in spinal motor neurons from patients with ALS, leading to a higher proportion of Q/R site-unedited GluR-containing Ca^{++} permeable AMPA receptors Kwak, S., *et al, Neuropathology* 30, 182-188, 2010).

[0008] Functional changes in AMPARs are most often attributed to phosphorylation events mediated by cyclic AMP-dependent protein kinase (PKA), protein kinase C (PKC) and CaM kinase II (calcium-calmodulin kinase II)(Greengard, P., *et al, Science* 253, 1135-8, 1991;

Wang, L. Y., *et al, J Physiol* 475, 431-7, 1994; Yakel, J. L., *et al,* Proc Natl Acad Sci U S A 92, 1376-80, 1995; Soderling, T. R., *Biochim Biophys Acta* 1297, 131-8, 1996; Barria, A., *et al, J Biol Chem* 272, 32727-30, 1997). Recently, a variety of intracellular proteins have been reported to bind directly to AMPARs (Xia, J., *et al, Neuron* 22, 179-87, 1999; Dong, H., *et al, Nature* 386, 279-84, 1997; Osten, P., *et al, Neuron* 21, 99-110, 1998; Daw, M. I., *et al, Neuron* 28, 873-86, 2000; Allison, D. W., *et al, J Neurosci* 18, 2423-36, 1998). These proteins play important roles not only in receptor targeting or clustering, but also in the modulation of receptor activity and activation of signaling pathways. Protein-protein interactions have been shown to affect AMPAR trafficking and function. The best characterized AMPAR interacting proteins are those that interact with the intracellular carboxyl terminus (CT) of AMPAR subunits such as GRIP (Glutamate Receptor Interacting Protein), ABP (AMPAR-binding protein), SAP97 (synapse-associated protein-97), PICKl (Protein interacting with C kinase-1), stargazin, NSF (N-ethylmaleimi de-sensitive factor), and AP2 (adaptor protein-2)(Xia *et al, supra;* Dong *et al, supra;* Osten *et al, supra;* Daw *et al, supra;* Chen, L., *et al, Nature,* 408(6815), 936-43, 2000; Lee, S.H., *et al, Neuron,* 36(4): 661-74, 2002; Nishimune, A., *et al, Neuron,* 21(1): 87-97, 1998; Song, I., *et al, Neuron,* 21(2): 393-400, 1998; Srivastava, S., *et al.*, Neuron, 21(3): 581-91, 1998; Dong, H., *et al, J Neurosci,* 19(16): 6930-41, 1999). These proteins have been shown to regulate AMPAR function in a variety of ways, including altering AMPAR localization, clustering and/or trafficking.

[0009] GAPDH is a tetrameric protein composed of four identical subunits (37 kDa). Each monomer has binding sites for the substrate (glyceraldehyde-3-phosphate, G3P) and the co-factor nicotinamide adenine dinucleotide (NAD^+)(Chuang, D. M., *et al, Annu Rev Pharmacol Toxicol* 45, 269-290, 2005; Sirover, M. A., *J Cell Biochem* 95, 45-52, 2005). Traditionally, GAPDH has been considered the key enzyme in glycolysis, and therefore, an important protein in energy production. In addition, GADPH was thought to be a housekeeping gene whose transcript level remained constant under most experimental conditions. However, recent evidence supports the notion that GAPDH plays a critical role in apoptosis, as indicated by changes in GAPDH expression and subcellular localization (Sawa, A., *et al, Proc Natl Acad Sci U S A*, 94(21): p. 11669-74, 1997; Ishitani, R., *et al, Mol Pharmacol,* 53(4): p. 701-7, 1998; Ishitani, R. and D.M. Chuang, *Proc Natl Acad Sci USA,* 93(18): 9937-41, 1996; Hara, M.R., *et al, Nat Cell Biol,* 2005. 7(7): 665-74, 2005). GAPDH is overexpressed and accumulates in the

nucleus during apoptosis induced by a variety of insults. The mechanism underlying GAPDH nuclear translocation and subsequent cell death remains largely unknown. However, recent studies have implicated several potential factors that may be involved in the process: (1) the expression of GAPDH is regulated by p53, a tumor suppressor protein and proapoptotic transcription factor, which suggests that GAPDH could be a downstream apoptotic mediator (Chen, R. W., *et al, J Neurosci* 19, 9654-9662, 1999); (2) overexpression of Bcl-2 blocks the apoptotic insults triggered by GAPDH overexpression, nuclear translocation and subsequent apoptosis, suggesting that Bcl-2 may participate in the regulation of GAPDH nuclear translocation, consistent with the anti-apoptic function of Bcl-2 (Dastoor, Z., and Dreyer, J. L., *J Cell Sci* 114, 1643-1653, 2001); (3) GAPDH binds to a nuclear localization-signal-containing protein, Siahl, which initiates its translocation to the nucleus. The association with GAPDH stabilizes Siahl and thereby enhances Siahl-mediated proteolytic cleavage of its nuclear substrates and triggers apoptosis (Hara *et al, supra;* Hara, M. R., and Snyder, S. H., *Annu Rev Pharmacol Toxicol.,* 2006; Hara, M. R., and Snyder, S. H., *Cell Mol Neurobiol,* 2006; Hara, M. R., *et al., Proc Natl Acad Sci USA* 103, 3887-3889, 2006).

[0010] Based on the foregoing, there is a need in the art for compositions and methods for the treatment and prophylaxis of MS.

SUMMARY OF THE INVENTION

[001 1] The present invention relates to compositions and methods for treatment and prophylaxis of MS.

[0012] According to a first aspect of an embodiment of the invention there is provided a polypeptide comprising SEQ ID NO:5. In a second aspect of a separate embodiment, the polypeptide does not comprise SEQ ID NO:l. In a third aspect of a separate embodiment, the polypeptide does not comprise a naturally occurring GluR2 subunit, for example, naturally occuring in the genome or proteome of any animal or subject that has not been genetically manipulated by man.

[0013] According to an aspect of the present invention there is provided a polypeptide defined by a fragment of SEQ ID NO:l that comprises 20 or more consecutive amino-acids thereof. For example, the polypeptide may comprise a fragment of SEQ ID NO:l which

comprises 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 consecutive amino acids of SEQ ID NO:l. According to a further aspect, there is provided a polypeptide that is at least 80 identical in sequence to any one of the above, for example, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Compositions that comprise the polypeptide as described above are also contemplated.

[0014] According to a further aspect of the present invention, there is provided a polypeptide comprising DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5) and wherein the polypeptide does not comprise SEQ ID NO:l.

[0015] In a further embodiment, the polypeptide comprises DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5), and is between 20 and 200 amino acids in length, for example, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 46, 48, 49, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 or more amino acids in length. The polypeptide may further comprise a range of lengths defined by any two values listed above, or any two values that lies between those specifically disclosed herein. Representative examples include, but without wishing to be considered limiting in any manner, 84 amino acids or a range of 21 to 157 amino acids.

[0016] In still a further embodiment, the polypeptide comprises DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5), but does not comprise SEQ ID NO.T or a fragment thereof, for example, any 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or 4 consecutive amino acids defined in SEQ ID NO:l.

[0017] In a further embodiment, which is not meant to be limiting, the polypeptide comprises SEQ ID NO: 5 but the C-terminal does not further comprise, in alternate embodiments, the amino acid defined by the one letter code A, R, N, D, B, C, E, Q, Z, G, H, I, L, K, M, F, P, S, T, W, Y or V. In still a further embodiment, which is not meant to be limiting in any manner the polypeptide comprises SEQ ID NO:5 but the N-terminal does not further comprise, in alternate separate embodiments, the amino acid defined by the one letter code A, R, N, D, B, C, E, Q, Z, G, H, I, L, K, M, F, P, S, T, W, Y or V. In yet a further embodiment, which is not meant to be limiting in any manner, the polypeptide comprises SEQ ID NO:5 but the N-terminal and C-terminal do not further each comprise individually, in alternate separate embodiments, an amino

acid defined by the one letter code A, R, N, D, B, C, E, Q, Z, G, H, I, L, K, M, F, P, S, T, W, Y or V. Also contemplated are occasions where the polypeptide C-terminus +2, +3, +4, +5, additional amino acids and/or the N-terminus +2, +3, +4, +5 additional amino acids in addition to SEQ ID NO:5 adhere to the above restrictions.

[001 8] Also contemplated herein are polypeptides that not identical to any GluR2 subunit that occurs in nature.

[0019] According to an aspect of the present invention, there is provided a method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering a polypeptide as defined above to a subject.

[0020] According to an another aspect of the present invention, there is provided a method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering a composition comprising the polypeptide as described to a subject.

[0021] According to a further aspect of the present invention, there is provided a method of increasing neuronal survival in a subject having multiple sclerosis, comprising administering a polypeptide as described above to a subject.

[0022] According to a yet further aspect of the present invention, there is provided a method of increasing neuronal survival in a subject having multiple sclerosis, comprising administering a composition comprising the polypeptide as described above to a subject.

[0023] According to an aspect of the present invention, there is provided a method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering a polypeptide of between 20 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l or SEQ ID NO:5 to a subject.

[0024] According to an another aspect of the present invention, there is provided a method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering a composition comprising polypeptide of between 20 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l or SEQ ID NO:5 to a subject.

[0025] According to a further aspect of the present invention, there is provided a method of increasing neuronal survival in a subject having multiple sclerosis, comprising administering a polypeptide of between 20 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l or SEQ ID NO:5 to a subject.

[0026] According to a yet further aspect of the present invention, there is provided a method of increasing neuronal survival in a subject having multiple sclerosis, comprising administering a composition comprising a polypeptide of between 20 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l or SEQ ID NO:5 to a subject.

[0027] In a non-limiting embodiment of the invention, the polypeptide consists of SEQ ID NO:l or SEQ ID NO:5. In a further non-limiting embodiment, the polypeptide is a fusion protein that comprises a protein transduction domain, for example, but not limited to the TAT sequence as is known in the art. In embodiments wherein SEQ ID NO:l or SEQ ID NO:5 are fused to a non-GluR2 subunit sequence (for example, a heterologous amino acid sequence) the fusion protein may be of any length provided it exhibits at least one desired activity as described herein.

[0028] The present invention also provides a polypeptide as defined above, that is attached covalently or non-covalently to a non-protein substrate, non-protein molecule, non¬protein macromolecule, a support, or any combination thereof. Further, the polypeptide, non-protein substrate, non-protein molecule, non-protein macromolecule, support or any combination thereof may be labelled by an appropriate group or marker as is known in the art and as described herein.

[0029] In an embodiment of the method of the present invention, the subject is a mammal. Preferably, the mammal is a primate, and more preferably, the primate is a human.

[0030] It is to be noted that the present invention also contemplates additional uses for the peptides as described herein and throughout.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

[0032] FIG. 1 shows nucleotide and amino acid sequences of polypeptides and nucleic as described herein. (A) shows the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l). (B) shows the amino acid sequence of Fragment 1 (Tyrl42 - Leul61) of the GluR2 N-terminus (SEQ ID NO:7) (C) shows the amino acid sequence of Fragment 2 (Aspl53 - Lysl72) of GluR2 N-terminus (SEQ ID NO:5). (D) shows a representative nucleotide sequence encoding a polypeptide that comprises the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:2). The shaded and underlined regions show a nucleotide sequence encoding residues Y142 to K172. (E) shows a polypeptide sequence of GluR2 comprising V22 to E545 (SEQ ID NO:3). The GluR2 NT1-3-2 (Y142-K172) amino acid sequence is underlined.

[0033] FIG.2 shows the characterization of the GluR2-GAPDH interaction in EAE animals. (A, B) shows coimmunoprecipitation of GAPDH with GluR2 from rat spinal cord tissues with a GluR2 antibody. PO.05, n=4 compared to control. (C) shows direct immunoprecipitation of GluR2 by GluR2 antibodies. Data are means ± SEM and are analyzed by t-test. (D, F) shows densitometric analysis of coimmunoprecipitation of GAPDH with GluR2 from mice spinal cord tissues with a GluR2 antibody in different treatment groups. (E) shows representative co-immunoprecipitation results of the interaction between GAPDH and GluR2 in the spinal cord of EAE modeling rats in different treatment groups. Blots and densitometry were done in triplicate. Data represents means ± s.e.m. Differences between groups were accessed by Student Newman-Keuls post-hoc ANOVA.

[0034] FIG. 3 represents a neurological assessment of EAE rats and the effect of treatment with TAT-G-Gpep. Treatments were started daily on day 10 after immunization. Rats were treated with TAT-G-Gpep, TAT-G-Gpep-sc until day 20.

[0035] FIG. 4 provides results of EAE modeled mice treated with TAT-G-Gpep (A) and TAT-G-Gpep-Sc (B).Clinical EAE scores (means±s.e.m) over time of four groups vaccinated with MOG35-55 on day 0 and treated intraperitoneally daily with TAT-G-Gpep and Scramble TAT G-Gpep from day 13 (arrow). Starting from Day 13, ($P<0.05$). Data was analyzed by two-way ANOVA.

[0036] FIG. 5 shows rescue of neuronal number in lumber spinal cord with TAT-G-Gpep peptide treatment. (A) represents Neu-N-immunostained fluroscence sections in saline, TAT-G-Gpep-sc treated and TAT-G-Gpep treated EAE rats shown at 5X magnification. (B) quantification of $NeuN^+$ cells in both dorsal and ventral horns revealed a significant increase in TAT-G-Gpep peptide-treated EAE rats (n=6-9) as compared to TAT-G-Gpep-sc treated EAE rats. Data are presented as means ± s.e.m. ** /?<0.01 vs saline.

[0037] FIG. 6 shows neuronal rescue with TAT-G-Gpep treatment in EAE mouse lumbar spinal cord. A, NeuN-immunostained image of lumbar spinal cord delineated into ventral, intermediate and dorsal zones. Scale Bar, IOOμm. B, Representative images of NeuN+ cells in sham, TAT-G-Gpep-Sc, non-treated and TAT-G-Gpep-treated mouse spinal cords. Scale Bar, IOOμηι. Quantification of C, total neurons and D, in dorsal, intermediate and ventral zones with different treatments. There were significantly fewer total neurons in both non-treated and peptide-treated mice spinal cords when compared to sham animals. Peptide-treatment resulted in a significantly more neuronal numbers in the intermediate and ventral zones vs. non-treated and TAT-G-Gpep-Sc-treated mice. All data are shown as mean ± SEM; * p<0.05, ** p<0.01 vs sham; ++ p<0.01.

[0038] FIG. 7 shows that TAT-G-Gpep treatment promotes oligodendrocytes survival in rat spinal cords with EAE. (A) represents CNPase-immunolabelled oligodendrocytes in sham, saline, non-treated and TAT-G-Gpep treated rat spinal cords. Scale bar, 20 μηι. (B) represents quantification of the number of CNPase-reactive oligodendrocytes in dorsal regions of rat spinal cords. All data are represented as mean ± s.e.m., $**p<0.01$ vs sham, $+p<0.05$.

[0039] FIG. 8 shows TAT-G-Gpep treatment promotes oligodendrocyte survival in mouse EAE spinal cord. Representative images of (A) CNPase-reactive and (C) CC1-immmunolabeled oligodendrocytes in sham, TAT-G-Gpep-Sc, non-treated and TAT-G-Gpep-treated mouse spinal cords. Scale Bar, 15μη. Quantification of the number of (B) CNPase- and (D), CC1-labeled oligodendrocytes in dorsal regions of mouse spinal cord. Oligodendrocyte numbers were significantly lower in scrambled peptide and non-treated mice when compared to sham, while peptide treatment significantly rescued oligodendrocyte numbers. All data are shown as mean ± SEM; $**p<0.01$ vs sham; ++/?<0.01 .

[0040] FIG. 9 shows that TAT-G-Gpep treatment rescues demyelination in EAE rat spinal cords. (A) shows Luxol Fast Blue staining of myelin (blue) in rat dorsal and ventral regions. Scale bar, 100 μηι. (B) represents quantification of myelin in both dorsal and ventral funiculus in rat spinal cords with different treatments. Images were converted to grey scale and calculated according to precalibrated values, and myelin density was measured in optical density. All data are represented as mean ± s.e.m., ** p<0.01 vs sham, + p<0.05, ++ p<0.01.

[0041] FIG. 10 shows that TAT-G-Gpep treatment rescues demyelination in EAE mouse spinal cord. (A) shows Luxol Fast Blue staining of myelin in mouse spinal cord and representative images of Luxol Fast Blue stains (gray scale) in sham, TAT-G-Gpep-Sc, non-treated and TAT-G-Gpep-treated mouse spinal cords. Scale bar, 50 μιη. (B) represents quantification of myelin in the ventral funiculus in mouse spinal cord with different treatments. Images were converted to grey scale and calculated according to precalibrated values, and myelin density was measured in optical density. Significant demylenation was observed in scrambled peptide and non-treated mice when compared to sham. TAT-G-Gpep treatment rsululted in significant rescue of myelination vs both scrambled peptide or non-treated groups. All data are represented as mean ± s.e.m., **/?<0.01 vs sham, ++ p<0.0\.

[0042] FIG. 11 shows that TAT-G-Gpep treatment rescues axonal density in rat spinal cord with EAE. (A) is a schematic diagram of coronal spinal cord section showing regions of interest in dorsal and ventral funiculus. (B) are representative images of neurofilament-H immunostained in sham, saline, non-treated and TAT-G-Gpep-treated rat spinal cords. Scale bar, 20 μηι. (C) represents quantification of the percentage area occupied by neurofilament-H in dorsal and ventral regions of rat spinal cords. All data are represented as mean ± s.e.m., ** p<0.01 vs sham, ++ p<0.0\.

[0043] FIG. 12 shows that TAT-G-Gpep treatment rescues axonal density in mice spinal cord with EAE. (A) is a schematic diagram of coronal spinal cord section showing regions of interest in dorsal and ventral horns of the grey matter. (B) are representative images of neurofilament-H immunostained in sham, saline, non-treated and TAT-G-Gpep-treated mouse spinal cords. Scale bar, 15 μη. (C) represents quantification of the percentage area occupied by neurofilament-H in mouse spinal cords. The area occupied by neurofilament-H was significantly

lower in scrambled peptide and non-treated mice when compared to sham, while peptide treated mice showed a significant rescue in axon density and was comparable to sham controls. All data are represented as mean ± s.e.m., **/?<0.01 vs sham, ++ p<0.01.

[0044] FIG. 13 represents axonal damage, assessed by Western blot for abnormally dephosphorylated neurofilament H. (A, C) show Western blot analysis of rat and mouse, respectively, whole spinal cord homogenate visualized by ECL. (B, D) show densitometric analysis of rat and mouse, respectively, of Western blots of total spinal cord homogenate of three representative animal per group, developed by HRP/DAB. Blots and densitometry were done in triplicate (Sham, TAT-G-Gpep-Sc, no treatment and TAT-G-Gpep). Data represents means ± s.e.m. Differences between groups were assessed by Student Newman-Keuls post-hoc one way ANOVA * p<0.05; ns, no significant difference.

[0045] FIG. 14 shows suppression of macrophages infiltration/microglia activation with TAT-G-Gpep treatment in EAE mouse spinal cord. (A) Representative images of Ibal-immunolabeled macrophages/microglia in sham, TAT-G-Gpep-Sc, non-treated and TAT-G-Gpep-treated mouse spinal cords. Scale Bar, 40μπι. (B) Quantification of the number of Ibal+ cells in the dorsal and ventral horns revealed significantly more macrophages/microglia residing in scrambled peptide, non-treated and peptide-treated mice when compared to sham. Peptide treatment significantly reduced the amount Ibal+ cells when compared to scrambled peptide or non-treated mice. All data were given as mean ± SEM; ** p<0.01 vs sham; + pO.01.

[0046] FIG. 15 (A) shows splenocytes from different groups that were harvested by the end of the disease course and cultivated in the presence of different concentration of MOG35-55 peptide for T cell proliferation. At low dose of MOG35-55 peptide (10μM), TAT-G-Gpep could suppress the T cell proliferation but not at high άθse(100μM). (B) Serum collected from different groups at the end of the disease course were tested for MOG-specific IgG2b levels. No differences were observed among different treatment groups. *P<0.05

[0047] FIG. 16 shows results suggesting TAT-GluR2nt 1-3-2 peptide but not the TAT-GluR2nt 1-3-2-scrm could block the infiltration of leukocytes into the CNS. (A) Representative FACS analysis of the BIL staining with CD4 and CD8. (B) Bar graph summarizing the FACS

analysis of BIL in different groups. (*P<0.05, comparing with other groups, ANOVA, post hoc SNK test).

[0048] FIG. 17 shows results suggesting TAT-GluR2nt 1-3-2 peptide but not the TAT-GluR2nt 1-3-2-scrm could inhibit number of CD4+ and CD8+ cells but not the CDl lb+ and CDl lc+ in spleenocytes. (A) Representative FACS analysis of the BIL staining with CD4 and CD8. (B) Bar graph summarizing the FACS analysis of CD4/CD8, CDl lb/CDllc in different groups. (*P<0.05, comparing with other groups, ANOVA, post hoc SNK test; ns, non¬ significant).

[0049] FIG. 18 shows results in bar graph form summarizing the Bioplex array results for cytokine profiles in the blood serum from different groups. No significant changes in the cytokine profile from mice blood serum in different groups were observed.

[0050] FIG. 19 shows results in bar graph form summarizing the Bioplex results for cytokine profiles in the cultured spleenocytes re-stimulated with MOG35-55 from different groups. For most cytokines, the immunoreactivity of spleenocytes from G-Gpep group is significantly suppressed comparing with the other two groups. (*P<0.05,**P<0.01, comparing with corresponding groups, ANOVA, post hoc SNK test).

[0051] FIG. 20 shows results of the pull-down affinity assay of GluR2-GAPDH Interaction. Three different fusion proteins were employed to precipitate GAPDH from hippocampal tissue homogenate. One of the fusion proteins was glutathione S-transferase alone (GST), which served as the negative control in this experiment. The other two fusion proteins were derived from two different fragments of the GluR2 N-terminus. Fragment 1 contained Tyrl42 - Leul61 amino acid residues of the GluR2 N-terminus (SEQ ID NO:7), while fragment 2 encoded an amino sequence of Aspl53 - Lysl72 (SEQ ID NO:5). Only the GST fusion protein containing fragment 2 was able to precipitate GAPDH (weight approximately 36kDa) proteins from solubilized rat hippocampal tissue among all three fusion proteins. For each of the pull¬ down assays presented above, a lane of pure rat hippocampal tissue homogeneate (70-100 μg) was included as positive control.

DETAILED DESCRIPTION

[0052] The present invention relates to polypeptides. The present invention also relates to polypeptides, compositions and methods for treatment and prophylaxis of MS.

[0053] The following description is of a preferred embodiment by way of example only and without limitation to the combination of features necessary for carrying the invention into effect.

[0054] In MS, an accumulation of glutamate is believed to be induced by activated immune cells, resulting in excessive stimulation of AMPAR, and eventually leading to loss of myelin, oligodendrocytes and some axons. Activation of AMPAR has been shown to cause the interaction between the GluR2 subunit of AMPAR and GAPDH (glyceraldehyde-3-phosphate dehydrogenase), a multifunctional protein that has a role in apoptosis. The polypeptide described herein has been previously shown to disrupt the GluR2-GAPDH interaction, and in rat models of global and focal ischemia, protects hippocampal neurons (PCT/CA2007/001539, the contents of which are incorporated herein by reference). As described herein, the G-Gpep polypeptide (also referred to as GluR2 NT1-3-2, Y142-K172 or SEQ ID NO:l herein) improves neurological function in EAE rat and mouse treated with G-Gpep fused to the cell-membrane transduction domain of HIV-1 virus TAT, referred to herein as TAT-G-Gpep. Moreover, administration of TAT-G-Gpep to EAE rats/mice mitigates neuronal death, rescues demyelination, increases oligodendrocyte survival and reduces damage in the spinal cord. A fragment of this polypeptide has also been shown to immunoprecipitate GAPDH from hippocampal tissue homogenate, similar to TAT-G-Gpep suggesting that this polypeptide fragment (SEQ ID NO:5) also may be capable of similar effects.

[0055] According to an aspect of the present invention there is provided a polypeptide comprising or consisting of SEQ ID NO:5, or a fragment of SEQ ID NO:l that comprises 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 consecutive amino acids of SEQ ID NO:l, but preferably not all of SEQ ID NO: 1. According to a further aspect, there is provided a polypeptide that is at least 80 identical in sequence to any one of the above, for example, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0056] According to a further aspect of the invention, administering a polypeptide as described above or herein, or a composition comprising the polypeptide as described above or

herein, for example, but not to be considered limiting in any manner, a polypeptide comprising SEQ ID NO: 5 or a polypeptide of between 31 and 200 amino acids comprising a GluR2 NT1-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l or fragment thereof to a subject can be used for the treatment or prophylaxis of multiple sclerosis.

[0057] As shown in FIG. 1, the polypeptides preferably used in the present method include: GluR2 NT1-3-2 (Y142-K172) represented by amino acid sequence (SEQ ID NO:l) or a fragment thereof, for example, a polypeptide comprising SEQ ID NO:5 as described herein, or a sequence which is at least 80% identical to SEQ ID NO:l, or a fragment thereof such as SEQ ID NO:5 that binds to GAPDH and wherein said polypeptide does not encompass a naturally occurring full length GluR2 subunit polypeptide.

[0058] As provided above, variations of the polypeptide sequence of SEQ ID NO:l are contemplated herein. For example, with respect to SEQ ID NO:l (GluR2 NT1-3-2), but not to be considered limiting in any manner, one or more residues at positions 3, 5, 18, 21, 22, 23, 26 or 30 of SEQ ID NO:l may be replaced by an alternate amino acid residue. For instance, but without wishing to be limiting, glutamine at position 3 may be replaced by another amino acid, for example, but not limited to lysine. Aspartic acid at position 5 may be replaced by another amino acid, for example, but not limited to threonine or glutamic acid. Serine at position 18 may be replaced by another amino acid, for example, but not limited to threonine. Glutamine at position 21 may be replaced by another amino acid, for example, but not limited to arginine. Alanine at position 22 may be replaced by another amino acid, for example, but not limited to valine or isoleucine. Valine at position 23 may be replaced by another amino acid, for example, but not limited to isoleucine. Serine at position 26 may be replaced by another amino acid, for example, but not limited to threonine. Lysine at position 30 may be replaced by another amino acid, for example, but not limited to arginine. These modifications have not been tested but are thought to provide the desired effect(s) as described herein. Other modifications are also possible and are contemplated herein. Further, the present invention contemplates variations wherein one or more of the replacements noted above are present in the polypeptide.

[0059] Naturally occurring full length GluR2 polypeptides and the sequences thereof are known in the art. For example, a search of the National Center for Biotechnology Information

using sequence information provided herein can be used to identify naturally occurring full length GluR2 protein sequences.

[0060] The present invention also provides for fragments of the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l), that do not encompass a naturally occurring full length GluR2 subunit, but rather is between about 20 and 200 amino acids in length, for example, but not limited to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 or any number of amino acids therein between. The present invention also encompasses polypeptides comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or a fragment thereof such as SEQ ID NO:5 that may be defined by a range of lengths of any two of the values provided above, or any values therein between. For example, but not to be limiting in any manner, the present invention provides a polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5 that is between 20 and 100, 20-200, 20-250, 25-200, 25-250, 27-300 amino acids in length, and the like.

[0061] The present invention also contemplates polypeptides having an amino acid sequence that comprises between about 80% to 100% sequence identity, for example, but not limited to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequences described above. Further, the polypeptides may be defined as comprising a range of sequence identities defined by any two of the values listed above.

[0062] The present invention also contemplates polypeptides that comprise fragments of GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l), for example 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, and 20 amino acids. Further, the present invention also contemplates fragments that exhibit at least about 80% identity, preferably 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to the polypeptides described above. The present invention also contemplates polypeptides that comprise fragments of GAPDH(2-2-l-l), for example 29, 28, 27, 26, 25, 24, 23, 22, 21, 20,19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, and 7 amino acids. The fragments may comprise N-terminal deletions, C-terminal deletions, internal deletions or any combination thereof.

[0063] It is also contemplated that the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5 may comprise part of a fusion protein, for example, but not limited to a polypeptide that further comprises a heterologous polypeptide or protein, for example, a carrier protein, a protein transduction domain or the like. For example, but not wishing to be limiting in any manner, the polypeptide of the present invention may be fused to a protein transduction domain to facilitate transit across lipid bilayers or membranes, for example, but not limited to as described in U.S. Publication 2002/0142299, U.S. Pat. No. 5,804,604, U.S. Pat. No. 5,747,641, U.S. Pat. No. 5,674,980, U.S. Pat. No. 5,670,617, and U.S. Pat. No. 5,652,122; PCT publication WO01/1551 1, US Publication 2004/0209797, PCT Publication W099/07728 ,US Publication 2003/0186890, all of which are herein incorporated by reference.

[0064] It is also contemplated that the polypeptide of the present invention may be attached either covalently or non-covalently to a non-protein substrate or molecule, for example, but not limited to polyethylene glycol (PEG), dextran or polydextran bead or the like, a support such as, but not limited to a multi-well plate, coverslip, array, micro-chip or the like. It is also contemplated that the polypeptide, non-protein substrate, molecule or any combination thereof may be labeled, for example with a purification tag, a radioactive or fluorescent group, enzyme or the like.

[0065] The present invention also provides nucleic acids encoding the polypeptides as described above. In an embodiment of the present invention which is not meant to be limiting, there is provided a nucleic acid encoding a polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NOT) or SEQ ID NO:5 that does not encode a naturally occurring full length GluR2 subunit. More preferably, but not wishing to be limiting in any manner, the present invention provides a nucleic acid encoding a polypeptide of between 20 and 200 amino acids and comprises the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NOT) or SEQ ID NO:5. An exemplary nucleic acid sequence, not meant to be limiting in any manner is shown FIG. IB as represented by SEQ ID NO:2.

[0066] The present invention also contemplates compositions comprising one or more of the polypeptides and/or nucleic acids of the present invention. The compositions may comprise one or more diluents, delivery vehicles, excipients, for example, but not limited to

pharmaceutically acceptable excipients as would be known in the art, buffers, media, solvents, solutions, carriers or the like. Such components alone or in any combination may provide a dosage form for using or administering the polypeptides or nucleic acids of the present invention to a solution, cell, cell culture, tissue, organ or subject, for example, but not limited to a human subject.

[0067] To determine whether a nucleic acid exhibits identity with the sequences presented herein, oligonucleotide alignment algorithms may be used, for example, but not limited to a BLAST (GenBank URL: www.ncbi.nlm.nih.gov/cgi-bin/BLAST/, using default parameters: Program: blastn; Database: nr; Expect 10; filter: default; Alignment: pairwise; Query genetic Codes: Standard(l)), BLAST2 (EMBL URL: http://www.embl-heidelberg.de/Services/ index.html using default parameters: Matrix BLOSUM62; Filter: default, echofilter: on, Expect: 10, cutoff: default; Strand: both; Descriptions: 50, Alignments: 50), or FASTA, search, using default parameters. Polypeptide alignment algorithms are also available, for example, without limitation, BLAST 2 Sequences (www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html, using default parameters Program: blastp; Matrix: BLOSUM62; Open gap (11) and extension gap (1) penalties; gap x_dropoff: 50; Expect 10; Word size: 3; filter: default).

[0068] An alternative indication that two nucleic acid sequences are substantially identical is that the two sequences hybridize to each other under moderately stringent, or preferably stringent, conditions. Hybridization to filter-bound sequences under moderately stringent conditions may, for example, be performed in 0.5 M $NaHP0_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.2 x SSC/0.1% SDS at 42°C for at least 1 hour (see Ausubel, et al. (eds), 1989, Current Protocols in Molecular Biology, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for example, be performed in 0.5 M $NaHP0_4$, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.1 x SSC/0.1% SDS at 68° C for at least 1 hour (see Ausubel, et al. (eds), 1989, supra). Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York). Generally, but not wishing to be

limiting, stringent conditions are selected to be about 5°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH.

[0069] A polypeptide of the invention can be synthesized *in vitro* or delivered to a cell *in vivo* by any conventional method. As a representative example of an *in vitro* method, the polypeptide may be chemically synthesized in vitro, or may be enzymatically synthesized *in vitro* in a suitable biological expression system. As a representative example of an *in vivo* method, a DNA, RNA, or DNA/RNA hybrid molecule comprising a nucleotide sequence encoding a polypeptide of the invention is introduced into an animal, and the nucleotide sequence is expressed within a cell of an animal.

[0070] The nucleotide sequence may be operably linked to regulatory elements in order to achieve preferential expression at desired times or in desired cell or tissue types. Furthermore, as will be known to one of skill in the art, other nucleotide sequences including, without limitation, 5' untranslated region, 3' untranslated regions, cap structure, poly A tail, translational initiators, sequences encoding signalling or targeting peptides, translational enhancers, transcriptional enhancers, translational terminators, transcriptional terminators, transcriptional promoters, may be operably linked with the nucleotide sequence encoding a polypeptide (see as a representative example "Genes VII", Lewin, B. Oxford University Press (2000) or "Molecular Cloning: A Laboratory Manual", Sambrook et al., Cold Spring Harbor Laboratory, 3rd edition (2001)). A nucleotide sequence encoding a polypeptide or a fusion polypeptide comprising the polypeptide may be incorporated into a suitable vector. Vectors may be commercialy obtained from companies such as Stratagene or InVitrogen. Vectors can also be individually constructed or modified using standard molecular biology techniques, as outlined, for example, in Sambrook et al. (Cold Spring Harbor Laboratory, 3rd edition (2001)). A vector may contain any number of nucleotide sequences encoding desired elements that may be operably linked to a nucleotide sequence encoding a polypeptide or fusion polypeptide comprising a protein transduction domain. Such nucleotide sequences encoding desired elements, include, but are not limited to, transcriptional promoters, transcriptional enhancers, transcriptional terminators, translational initiators, translational terminators, ribosome binding sites, 5' untranslated region, 3' untranslated regions, cap structure, poly A tail, origin of replication, detectable markers, afffinity tags, signal or target peptide, Persons skilled in the art will recognize that the selection and/or construction

of a suitable vector may depend upon several factors, including, without limitation, the size of the nucleic acid to be incorporated into the vector, the type of transcriptional and translational control elements desired, the level of expression desired, copy number desired, whether chromosomal integration is desired, the type of selection process that is desired, or the host cell or the host range that is intended to be transformed.

[0071] As described herein, and unless clearly indicated otherwise, the term "mini-gene" means the expression product of a nucleic acid or nucleotide sequence encoding and capable of expressing a polypeptide in a cell. For example, but not wishing to be considered limiting in any manner, a mini-gene includes a nucleic acid or nucleotide sequence encoding and capable of expressing the polypeptide, for example, the polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5 in a cell.

[0072] The DNA, RNA, or DNA/RNA hybrid molecule may be introduced intracellularly, extracellularly into a cavity, interstitial space, into the circulation of an organism, orally, or by any other standard route of introduction for therapeutic molecules and/or pharmaceutical compositions. Standard physical methods of introducing nucleic acids include, but are not limited to, injection of a solution comprising RNA, DNA, or RNA/DNA hybrids, bombardment by particles covered by the nucleic acid, bathing a cell or organism in a solution of the nucleic acid, or electroporation of cell membranes in the presence of the nucleic acid.

[0073] A nucleic acid may be introduced into suitable eukaryotic cells *ex vivo* and the cells harbouring the nucleic acid can then be inserted into a desired location in an animal. A nucleic acid can also be used to transform prokaryotic cells, and the transformed prokaryotic cells can be introduced into an animal, for example, through an oral route. Those skilled in the art will recognize that a nucleic acid may be constructed in such a fashion that the transformed prokaryotic cells can express and secrete a polypeptide of the invention. Further, a nucleic acid may also be inserted into a viral vector and packaged into viral particles for efficient delivery and expression.

[0074] The polypeptides of the present invention or the nucleic acids encoding the polypeptides of the present invention may be formulated into any convenient dosage form as would be known in the art. The dosage form may comprise, but is not limited to an oral dosage

form wherein the agent is dissolved, suspended or the like in a suitable excipient such as but not limited to water or saline. In addition, the agent may be formulated into a dosage form that could be applied topically or could be administered by inhaler, or by injection either subcutaneously, into organs, or into circulation. An injectable dosage form may include other carriers that may function to enhance the activity of the agent. Any suitable carrier known in the art may be used. Also, the agent may be formulated for use in the production of a medicament. Many methods for the productions of dosage forms, medicaments, or pharmaceutical compositions are well known in the art and can be readily applied to the present invention by persons skilled in the art.

[0075] According to the present invention there is also provided a method of inhibiting GluR2 subunit association with GAPDH comprising: administering a polypeptide as described herein, for example a polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5 to a solution, cell, cell culture, tissue or subject comprising GluR2 subunit and GAPDH. The method may be practiced *in vitro* or *in vivo.* In an embodiment wherein the method is practiced *in vivo,* the method may be practiced in a human subject. The human subject may have or be susceptible to multiple sclerosis.

[0076] In still a further embodiment of the present invention, which is not meant to be limiting in any manner, there is provided a method for treatment or prophylaxis of multiple sclerosis comprising, administering: a polypeptide as described herein, for example, a polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5; or a nucleic acid capable of expressing a polypeptide comprising the GluR2 NT1-3-2 (Y142-K172) amino acid sequence (SEQ ID NO:l) or SEQ ID NO:5 to a subject in need thereof. As will be evident to a person of skill in the art, an embodiment that comprises administering a nucleic acid as described above, further comprises the step of expressing nucleic acid in the subject.

[0077] It follows from the description above that the present invention also contemplates a polypeptide as described herein for use in the prophylaxis and/or treatment of neurological conditions such as MS in a subject. Other uses as described herein are also contemplated as are uses for the treatment of, or for the production of a medicament for the prophylaxis and/or treatment of neurological conditions such as MS in a subject.

[0078] The present invention also contemplates a method as defined above wherein the polypeptide is administered prior to, during, after or both prior to and after an event that is associated with multiple sclerosis, for example, but not limited to weakness, fatigue, loss of vision, cognitive impairment and impaired balance and coordination. For example, but not to be considered limiting in any manner, subjects diagnosed with MS may be administered the polypeptide of the present invention at one or more intervals after being diagnosed with the condition, preferably prior to, during or after an MS episode. In another embodiment, the polypeptide is administered as prophylactic treatment to individuals being genetically predisposed to the development of MS or have had one or more MS episodes in the past and are at risk to having another episode.

[0079] **EXAMPLES**

[0080] General Methodology

[008 1] Animals model and tissue harvest

[0082] C57/B16 mice (4-6 weeks old) used in this study were all purchased from Charles Rivers. All animal studies were approved by the Canada Animal Care Committee. Briefly, EAE was induced in C57/B16 mice by subcutaneous (s.c.) immunization with 200 μg of the murine myelin oligodendrocyte glycoprotein (MOG) peptide 35-55 (MEVGWYRSPFSRVVHLYRNGK) SEQ ID NO:6 emulsified in 100 μï of complete Freund's Adjuvant. Two hundreds ng of pertussis toxin in 100 μï of phosphate-buffered saline (PBS) were injected i.p. on the day of immunization (day 0) and two days later. ImM GluR2ntl-3-2 peptide was injected intreperitonally (i.p.) daily starting on day 10 after immunization. Control mice received GluR2NTl-3-2-SCRM (scrambled GluR2NTl-3-2 polypeptide) in the same concentration.

[0083] The clinical score was assessed daily from day 1 after the immunization. a 0-4 scoring system was used: 0, no obvious signs; 0.5, Distal paresis of the tail; 1, Complete tail paralysis; 1.5, Paresis of the tail and mild hind leg paresis; 2, unilateral severe hind leg paresis; 2.5, bilateral severe hind limb paresis; 3, complete bilateral hind limb paralysis; 3.5, complete

bilateral hind limb paralysis and paresis of one front limb; 4, complete paralysis (tetraplegia), moribund state, or death.

[0084] At day 30, mice were sacrificed by overdose anesthesia, and the spinal cord was dissected for co-immunoprecipitation analysis. For analysis of central nervous system infiltrates, brain and spinal cord tissues were collected and mononuclear cells were prepared by Percoll gradient centrifugation. For immunological analysis, splenocytes cells were dissected and cultured *in vitro.* For histological analysis, the same tissue samples were immediately fixed in 4% (wt/vol) paraformaldehyde. Frozen sections of spinal cord were stained with Luxol fast blue for analysis of inflammation and demyelination, respectively.

[0085] Lymphocytes proliferation Assay

[0086] Lymphocytes of the EAE mice in different treatment groups were obtained from mice spleen. Briefly, mice were sacrificed and spleen were dissected. Spleen tissues were placed in DMEM medium and cut into small pieces ($1mm^3$), and then passed through a 40μηι cell strainer in DMEM medium. Red blood cell lysis buffer was used to remove all the red blood cells. After centrifugation, single cell suspensions were cultured in 96 well plates and stimulated with MOG 35-55 peptide in different concentrations (0 μM, 10 μM, IOOμM) or PHA (5 μM) for 72h. A 10 mL aliquot containing 1 mCi 3H-thymidine was added prior to the final 18 h of culture. Cells described above were harvested onto glass-fiber filters for the assay of radioactivity by a liquid β-scintillation counter (Perkin-Elmer, Wellesley, MA, USA).

[0087] Bioplex Protein Array

[0088] Cytokine measurement was performed by Bioplex Protein Array (Bio-Plex Pro™ Mouse Cytokine 23-plex Assay) from Bio-Rad as per manufacturer's instruction. EAE mice serum, culture supernatant of spleenocytes in different treatment groups and supernatant of brain infiltrated leukocyte culture were used for bioplex protein array in duplicates. The standard curves were optimized automatically by the software (Bioplex manager) and verified manually. The Bioplex manager software was used to calculate cytokine concentrations. Data is expressed as Mean ± SEM.

[0089] Immunofluoresence

[0090] Frozen coronal sections of EAE mice spinal cord (10 μm) thickness were cut using a microtome cryostat system (Bright Instruments 5030). All sections were initially incubated in blocking solution (0.1 m PBS, 1% Triton X-100, 0.5% Tween 20, 2% skim milk) or serum-free protein block (Dako Cytomation) for 1 h at room temperature to reduce nonspecific background and then incubated with primary and secondary antibodies overnight at 4°C. The following primary antibodies were used: anti-Ibal (1:200; Dako), anti-Neurofilament H (1:200), anti-CCl, anti-CNPase (1:200), and anti-NeuN (1:200). Fluorescent secondary antibodies conjugated to Alexa 488 or Rhodamine Red-X (1:200; Invitrogen) or Cy3 (1:100; Jackson ImmunoResearch Laboratories) were used for detection of primary antibodies.

[0091] Flow cytometry

[0092] Flow cytometry was conducted using spleenocytes obtained from different groups. Briefly, cells were incubated with anti-CD4(FITC BD), and anti-CD8(Cy7, BD) or anti-CDl lb(PE, BD) and anti-CDl lc (Cy7, BD) for lh at room temperature. After washing, cells was fixed with fixation buffer (BD) for 30min and filtered using 80μm cell strainer. Samples were analyzed within 24 h with BD FACScan (BD Biosciences) using Cell Quest software (BD Biosciences). Isotypematched, PE- and FITC-conjugated mAb of irrelevant specificity were tested as negative controls.

[0093] **Example 1: GIuR2-GAPDH interaction is increased in the spinal cord of EAE rats and mice**

[0094] To investigate whether the GluR2-GAPDH interaction plays a role in the pathology of MS, GluR2-GAPDH complex formation was tested to determine if the formation is altered in the spinal cord of EAE rats. EAE was induced by immunizing Lewis rats with guinea pig myelin basic protein emulsified in Freund's complete adjuvant containing *Mycobacterium tuberculosis.* Neurological decline was observed beginning 9-1 1 days after immunization, consistent with previous reports (Schluesener, H.J., *et al, J Neuroimmunol* 18, 341-351, 1988). Rats were sacrificed on day 14 (previous studies indicate that the neurological decline reaches a peak on day 13 after immunization), and spinal cord protein was extracted to examine the interaction between GluR2 and GAPDH by co-immunoprecipitation. Consistent with the results from rat ischemia models, GluR2-GAPDH interaction was increased in EAE rat spinal cord

protein extract in comparison to control rats, by using GluR2 antibody to co-immunoprecipitate GAPDH (Figure 2A, 2B) (n=4, $p<0.05$; Data are means ±SEM. and are analyzed by t-test). There was no difference in directly-immunoprecipitated GluR2 levels between EAE and control rats (Figure 2C). Similar results were also obtained in EAE mice (Figure 2D,F). Mouse EAE was induced using **$MOG35_{-55}$** emulsified with CFA. These mice started to develop MS-like symptoms on day 10. At day 13, treatment of mice daily was started with either TAT-G-Gpep or TAT-G-Gpep-Sc (LP.). Mice were sacrificed on day 28 for extraction of spinal cord protein. The interaction between GluR2 and GAPDH was examined by co-immunoprecipitation, and an enhanced GluR2-GAPDH interaction was found in EAE mice. Injection of TAT-G-Gpep was able to disrupt the GluR2-GAPDH interactions.

[0095] **Example 2: Disruption of GluR2-GAPDH interaction improved neurological function in EAE rats and mice**

[0096] An interfering peptide (G-Gpep) also known as GluR2 NT1-3-2 having the amino acid sequence YYQWDKFAYLYDSDRGLSTLQAVLDSAAEKK (SEQ ID NO:l) capable of disrupting GluR2-GAPDH interactions and protecting neurons from ischemia-induced cell death in animal models of ischemic stroke was used. A scrambled G-Gpep peptide (G-Gpep-sc or G-Gpep-scrambled) having the amino acid sequence YGRKKRRQRRRAFDLSQYDLKWQVDYLKYDYGTASELRASA (SEQ ID NO:4) was used as a control peptide. To enable all peptides to permeate the cell membrane, they are fused to the cell-membrane transduction domain of the HIV-type 1 virus: TAT domain. Intraperitoneal (IP) injection of TAT-G-Gpep was chosen in the current experiment due to the requirement for repeated injections in the EAE models. The daily peptide injection regimen was started on the tenth day after rats are immunized and the 13^{th} day after mice are immunized, based on the previous studies on AMPAR antagonists in the treatment of MS (Pitt, D., *et al, Nat Med* 6, 67-70, 2000; Smith, T., *et al, Nat Med* 6, 62-66, 2000; Kanwar, J.R., *et al, Brain* 127, 1313-1331, 2004). The neurological score was rated each day, one hour after peptide injection. As shown in Figures 3 (rats) and 4 (mice), IP application of TAT-G-Gpep (3nmol/g) daily to the immunized animals significantly reduced the cumulative neurological score compared to EAE rat/mice without peptide or TAT-G-Gpep-sc treated EAE rats/mice.

Example 3: Disruption of GluR2-GAPDH interaction mitigated AMPAR toxicity-associated injury in the spinal cord of EAE rats/mice

[0097] Loss of myelin, oligodendrocytes and some axons are core features of MS pathology. As such, spinal cord sections from the lumbar region of EAE rats and mice treated with TAT-G-Gpep peptide were examined in comparison to TAT-G-Gpep-sc as a control.

[0098] IP injection of TAT-G-Gpep was found to enhance spinal cord neuron density as indexed by counting $NeuN^+$ cells in dorsal and ventral horns of the lumbar region of EAE rats (Figure 5)/mice (Figure 6) compared to EAE rats/mice treated with TAT-G-Gpep-sc.

[0099] IP injection of TAT-G-Gpep was also found to promote oligodendrocyte survival as indexed by CNPase-immunolabeled oligodendrocytes in spinal cord of EAE rats (Figure 7)/mice (Figure 8) compared to EAE rats/mice treated with TAT-G-Gpep-sc.

[00100] Luxol Fast Blue staining of myelin indicated that IP injection of TAT-G-Gpep rescues demyelination in the spinal cord of EAE rats (Figure 9)/mice (Figure 10) compared to EAE rats/mice treated with TAT-G-Gpep-sc.

[00101] IP injection of TAT-G-Gpep also rescues axonal density in the spinal cord of EAE rats/mice compared to EAE rats/mice treated with TAT-G-Gpep-sc. As shown in Figure 11 (rats) and Figure 12 (mice), immune-labeled neurofilament-H was significantly increased in the spinal cord of EAE rats/mice treated with TAT-G-Gpep compared to EAE rats/mice treated with TAT-G-Gpep-sc. Furthermore, axonal damage was assessed by Western Blot for abnormally dephosphorylated neurofilament H. TAT-G-Gpep treatment significantly reduced dephosphorylated neurofilament H in EAE rats/mice compared with TAT-G-Gpep-sc (Figure 13).

Example 4: Disruption of GluR2-GAPDH interaction with TAT-G-Gpep suppressed macrophage/microglia infiltration in EAE rat/mice spinal cord

[00102] As MS is closely associated with inflammation and autoimmunity, the TAT-G-Gpep peptide was tested to determine if it affects microglial/macrophage recruitment. As shown in Figure 14, the number of $Ibal^+$ cells in mouse spinal chord was differentially increased in

EAE treatment groups when compared to sham controls and there was a significant difference in Ibal-immunolabeled macrophages/microglia among TAT-G-Gpep-sc, TAT-G-Gpep treated and non-treatment groups. These data suggested that the interfering peptide TAT-G-Gpep has an effect on activation of the immune system in the spinal cord of EAE animals.

Example 5: 20 amino acid GluR2 N-terminal fragment (SEQ ID NO:5) precipitates GAPDH from solublized rat hippocampal tissue

[00 103] Production of GST-Fusion Proteins

[00104] Desired DNA sequences of the GluR2 N-terminus were obtained by polymerase chain reaction. The DNA sequences were digested with BamHI and Xhol enzymes to create sticky ends and subsequently incorporated into GST-plasmids (pGEX4T3) through DNA ligation. Following ligation, transformation with DH5a competent cells was performed to amplify the plasmid content. Subsequently, the plasmids were extracted and subjected to sequence confirmation. Having the desired DNA sequences confirmed, the GST-fusion proteins were expressed through the transformation with another strain of competent cells (i.e. BL21). The GST-fusion proteins were then precipitated and purified through glutathione resin (from Genescript).

[00105] Pull-Down Affinity Assay

[00106] Rat hippocampus was homogenized in 50 mM Tris-HCl (pH 7.5), 150 raM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA, 10% Triton X-100, 1 mM PMSF and protease inhibitor cocktail (Sigma, 5 microl per 100 mg tissue). The hippocampal tissue was further solubilized with 1% Triton X-100 and then centrifuged at 14,000rpm at 4 °C for 15 mins. The solubilized hippocampal tissue (approximately 1 milligram of protein) was incubated with glutathione resin (Genescript) and indicated GST-fusion proteins (approximately 100μg) at 4 °C overnight and followed by an hour of incubation at room temperature. After the incubation, the samples were washed 4-6 times with 1 mL PBS contained 0.1% Triton X-100. Following the series of washing, 30μL of glutathione elution buffer was added to elute the proteins from the glutathione beads. The eluates were incubated in sample buffer (final concentration 5% SDS), boiled for 5 minutes and eventually subjected to SDS-PAGE (12% gel). The blots were blocked

with 5% non-fat milk dissolved in TBST buffer (10 mM Tris-HCl, 150 raM NaCl and 0.1% Tween) for 1 h at room temperature. Prior to primary antibody incubation, the blots were washed three times with TBST to remove any milk residues. The blots were then incubated with primary antibodies against GAPDH (diluted 1:500, Millipore) at 4°C overnight. On the next day, GADPH were revealed with peroxidase-conjugated secondary antibodies (Sigma) and enhanced chemiluminescence (GE Healthcare UK Limited).

[00107] Three different GST-fusion proteins were tested in this pull-down affinity assay. They were respectively GST alone, GST-Fragment 1 (wherein fragment 1 is Tyrl42 - Leul61 of GluR2 N-terminus; YYQWDKFAYLYDSDRGLSTL (SEQ ID NO:7)) and GST-Fragment 2 (wherein fragment 2 is Aspl53 - Lysl72 of GluR2 N-terminus; DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5)) fusion proteins. As observed in Figure 20, neither the GST alone nor the GST-fusion protein containing fragment 1 (Tyrl42 - Leul61 (SEQ ID NO:7)) sufficed to "pull down" GAPDH from the solubilized hippocampal tissue. On the other hand, the GST-fusion protein containing fragment 2 (Asp 153 - Lysl72 (SEQ ID NO: 5)) of the GluR2 N-terminus was able to precipitate GAPDH, which weighs approximately 36 kDa. It is worth noting that the input lane was designated as the positive control and its GAPDH band perfectly aligned with the one in the GST-fragment 2 lane, further confirming the successful precipitation by GST-fragment2 fusion protein. Based on this finding, but without wishing to be bound by any particular theory or limiting in any manner, it is clear that the 20 amino acid sequence of fragment 2 was important in facilitating the protein interaction between GluR2 and GAPDH.

[00108] The present invention has been described with regard to one or more embodiments. However, it will be apparent to persons skilled in the art that a number of variations and modifications can be made without departing from the scope of the invention as defined in the claims.

[00109] The disclosure provided contains statements that are based on experimental results provided herein. The disclosure may also comprise statements that are forward looking and/or predicted based on the results presented herein and should not be taken as promises of the

invention. Finally, the application comprises disclosure for the purpose of providing literal support for claim amendments in response to unknown prior art.

[001 10] All citations are herein incorporated by reference.

CLAIMS

1. A polypeptide comprising Asp 153 - Lys 172 of the GluR2 N-terminus (DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5)) and wherein the polypeptide does not comprise SEQ ID NO:l.

2. The polypeptide of claim 1, further comprising a protein transduction domain.

3. The polypeptide of claim 1, wherein said polypeptide is covalently attached to a heterologous polypeptide, to form a fusion protein, wherein said heterologous polypeptide does not encompass a GluR2 subunit sequence.

4. The polypeptide of claim 1, said polypeptide attached covalently or non-covalently to a non-protein substrate, non-protein molecule, non-protein macromolecule, a support, or any combination thereof.

5. A composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier, diluent or excipient.

6. A nucleotide sequence encoding the polypeptide of claim 1.

7. A method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering the polypeptide of claim 1 or a polypeptide of between 31 and 200 amino acids comprising a GluR2 NT 1-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l to a subject.

8. The method of claim 7, wherein said polypeptide consists of SEQ ID NO:1 or SEQ ID NO:5.

9. The method of claim 7, wherein said polypeptide is covalently attached to a heterologous polypeptide, to form a fusion protein.

10. The method of claim 6, wherein said fusion protein comprises a protein transduction domain.

11. The method of claim 7, wherein said polypeptide is attached covalently or non-covalently

to a non-protein substrate, non-protein molecule, non-protein macromolecule, or any combination thereof.

12. The method of claim 11, wherein the polypeptide, non-protein substrate, non-protein molecule, non-protein macromolecule, or any combination thereof is labelled.

13. The method of claim 7, wherein the subject is a human.

14. A method for treatment or prophylaxis of multiple sclerosis in a subject, comprising administering a composition comprising the polypeptide of claim 1, or a composition comprising a polypeptide of between 31 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO: 1 to a subject.

15. The method of claim 14, wherein said polypeptide consists of SEQ ID NO: 1 or SEQ ID NO:5.

16. The method of claim 14, wherein said polypeptide is covalently attached to a heterologous polypeptide, to form a fusion protein.

17. The method of claim 14, wherein said fusion protein comprises a protein transduction domain.

18. The method of claim 14, wherein said polypeptide attached covalently or non-covalently to a non-protein substrate, non-protein molecule, non-protein macromolecule, or any combination thereof.

19. The method of claim 18, wherein the polypeptide, non-protein substrate, non-protein molecule, non-protein macromolecule, or any combination thereof is labelled.

20. The method of claim 14, wherein the subject is a human.

21. A method of increasing neuronal survival in a subject having multiple sclerosis, comprising administering the polypeptide of claim 1 or a polypeptide of between 31 and 200 amino acids comprising a GluR2 NTl-3-2 (Y142-K172) amino acid sequence defined by SEQ ID NO:l to a subject.

22. The method of claim 18, wherein said polypeptide consists of SEQ ID NO: 1 or SEQ ID NO:5.

FIGURE 1A: GluR2 NT1-3-2

YYQWDKFAYLYDSDRGLSTLQAVLDSAAEKK (SEQ ID NO:1)

FIGURE 1B: Fragment 1 (Tyr142 – Leu161) of GluR2 N-terminus
YYQWDKFAYLYDSDRGLSTL (SEQ ID NO:7)

FIGURE 1C : Fragment 2 (Asp153 – Lys172) of GluR2 N-terminus;
DSDRGLSTLQAVLDSAAEKK (SEQ ID NO:5)

FIGURE 1D:DNA sequence of GluR2 amino terminus:

1 atgcaaaaga ttatgcatat ttctgtcctc ctttctcctg ttttatgggg actgattttt
61 ggtgtctctt ctaacagcat acagataggg gggctatttc caaggggcgc tgatcaagaa
121 tacagtgcat ttcgggtagg gatggttcag ttttccactt cggagttcag actgacaccc
181 catatcgaca atttggaggt agccaacagt ttcgcagtca ccaatgcttt ctgctcccag
241 ttttcaagag gagtctacgc aatttttgga ttttatgaca agaagtctgt aaataccatc
301 acatcattct gtgggacact ccatgtgtcc ttcatcacac ctagcttccc aacagatggc
361 acacatccat ttgtcatcca gatgcgacct gacctcaaag gagcactcct tagcttgatt
421 gagtactacc aatgggacaa gttcgcatac ctctatgaca gtgacagagg cttatcaaca
481 ctgcaagctg ttctggattc tgctgcagag aagaagtggc aggtgactgc tatcaatgtg
541 gggaacatca acaatgacaa gaaagatgag acctacagat cgctctttca agatctggag
601 ttaaaaaaag aacggcgtgt aatcctggac tgtgaaaggg ataaagtaaa tgacattgtg
661 gaccaggtta ttaccattgg aaaacatgtt aaagggtacc attatatcat tgcaaatctg
721 ggattcactg atggggacct gctgaaaatt cagtttggag gagcaaatgt ctctggattt
781 cagattgtag actacgatga ttccctggtg tctaaattta tagaaagatg gtcaacactg
841 gaagagaaag aataccctgg agcacacaca gcgacaatta agtatacttc ggccctgacg
901 tatgatgctg tccaagtgat gactgaagca ttccgtaacc ttcggaagca gaggattgaa
961 atatcccgga gaggaaatgc aggggattgt ttggccaacc cagctgtgcc ctggggacaa
1021 ggggtcgaaa tagaaagggc cctcaagcag gttcaagttg aaggcctctc tggaaatata
1081 aagtttgacc agaatggaaa acgaataaac tacacaatta acatcatgga gctcaaaaca
1141 aatggacccc ggaagattgg gtactggagt gaagtggata aaatggttgt caccctaact
1201 gagctcccat caggaaatga cacgtctggg cttgaaaaca agactgtggt ggtcaccaca
1261 atattggaat ctccatatgt tatgatgaag aaaaatcatg aaatgcttga agggaatgag
1321 cgttacgagg gctactgtgt tgacttagct gcagaaattg ccaaacactg tgggttcaag
1381 tacaagctga ctattgttgg ggatggcaag tatggggcca gggatgccga caccaaaatt
1441 tggaatggta tggttggaga gcttgtctac gggaaagctg acattgcaat tgctccatta
1501 actatcactc tcgtgagaga agaggtgatt gacttctcca agcccttcat gagtcttgga
1561 atctctatca tgatcaagaa gcctcagaag tccaaaccag gagtgttttc ctttcttgat
1621 cctttagcct atgag (SEQ ID NO:2)

The shaded and underlined region shows a representative sequence encoding GluR2 NT1-3-2 (Y142-K172).

FIGURE 1E: V22-E545 of GluR2 (GluR2 NT1-3-2 is underlined)

$GluR_2NT$ V_{22}-E_{545}

22 31 41 51
VSSNSIQIG GLFPRGADQE YSAFRVGMVQ FSTSEFRLTP
61 71 81 91
HIDNLEVANS FAVTNAFCSQ FSRGVYAIFG FYDKKSVNTI
101 111 121 131
TSFCGTLHVS FITPSFPTDG THPFVIQMRP DLKGALLSLI
141 151 161 171
EYYQWDKFAY LYDSDRGLST LQAVLDSAAE KKWQVTAINV
181 191 201 211
GNINNDKKDE TYRSLFQDLE LKKERRVILD CERDKVNDIV
221 231 241 251
DQVITIGKHV KGYHYIIANL GFTDGDLLKI QFGGANVSGF
261 271 281 291
QIVDYDDSLV SKFIERWSTL EEKEYPGAHT ATIKYTSALT
301 311 321 331
YDAVQVMTEA FRNLRKQRIE ISRRGNAGDC LANPAVPWGQ
341 351 361 371
GVEIERALKQ VQVEGLSGNI KFDQNGKRIN YTINIMELKT
381 391 401 411
NGPRKIGYWS EVDKMVVTLT ELPSGNDTSG LENKTVVVTT
421 431 441 451
ILESPYVMMK KNHEMLEGNE RYEGYCVDLA AEIAKHCGFK
461 471 481 491
YKLTIVGDGK YGARDADTKI WNGMVGELVY GKADIAIAPL
501 511 521 531
TITLVREEVI DFSKPFMSLG ISIMIKKPQK SKPGVFSFLD
541
PLAYE (SEQ ID NO:3)

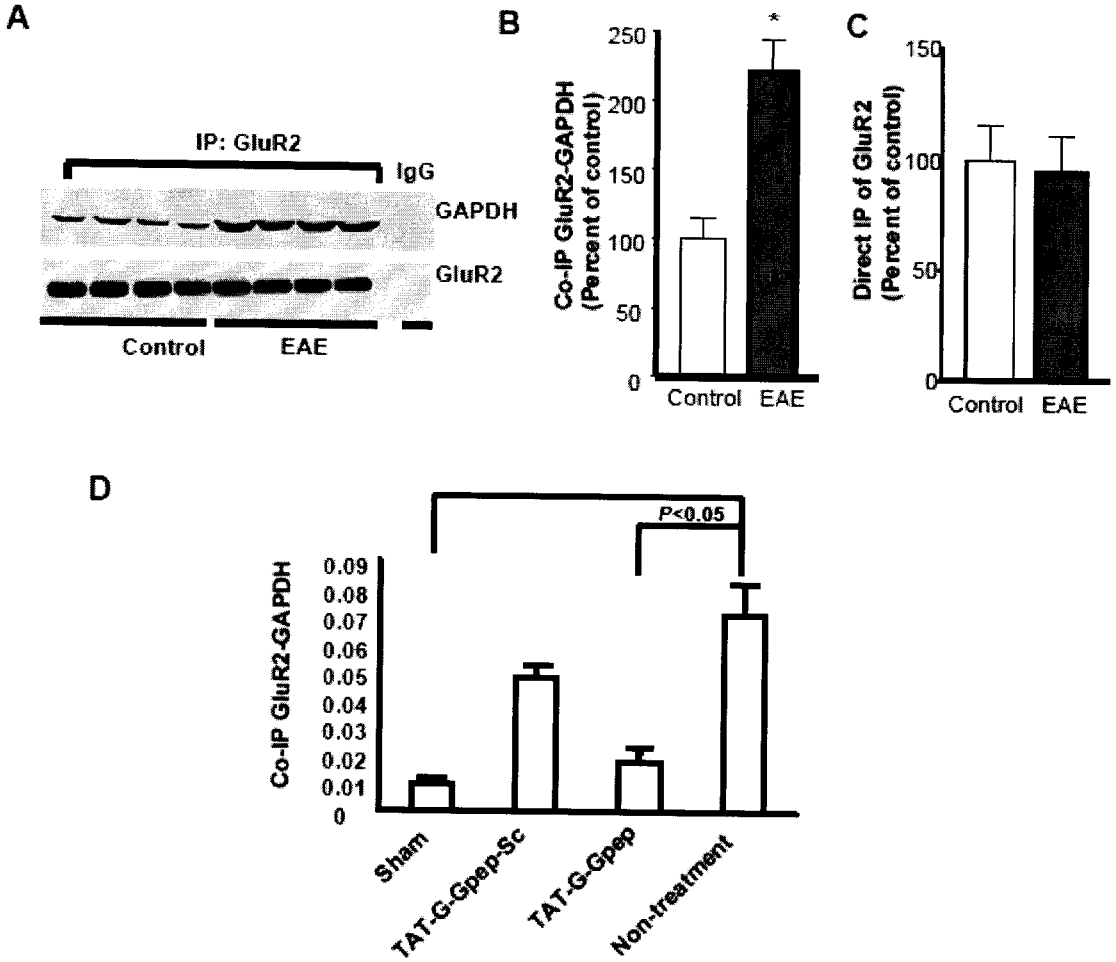
A
IP: GluR2
IgG
GAPDH
GluR2
Control
EAE
B
Co-IP GluR2-GAPDH (Percent of control)
250
200
150
100
50
0
*
Control
EAE
C
Direct IP of GluR2 (Percent of control)
150
100
50
0
Control
EAE
D
P<0.05
Co-IP GluR2-GAPDH
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Sham
TAT-G-Gpep-Sc
TAT-G-Gpep
Non-treatment

FIG. 2

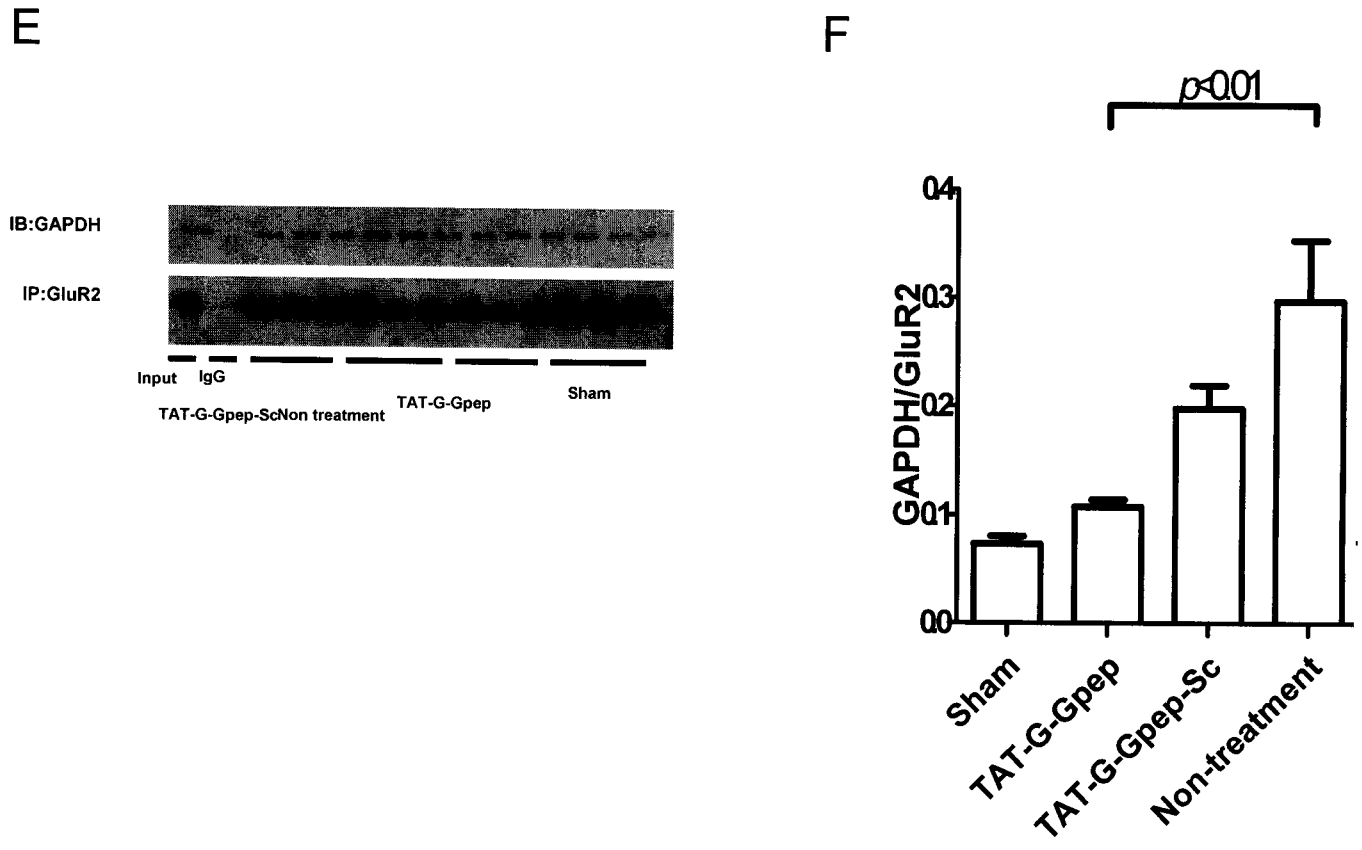
E
IB:GAPDH
IP:GluR2
Input
IgG
TAT-G-Gpep-ScNon treatment
TAT-G-Gpep
Sham
F
p<0.01
GAPDH/GluR2
0.4
0.3
0.2
0.1
0.0
Sham
TAT-G-Gpep
TAT-G-Gpep-Sc
Non-treatment

FIG. 2 Cont'd

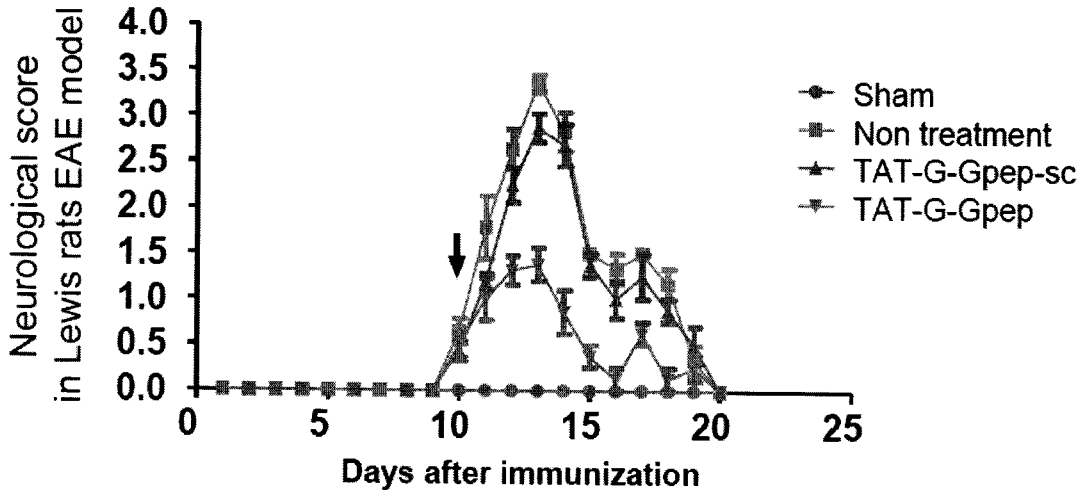
Neurological score
in Lewis rats EAE model
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Days after immunization
Sham
Non treatment
TAT-G-Gpep-sc
TAT-G-Gpep

FIG. 3

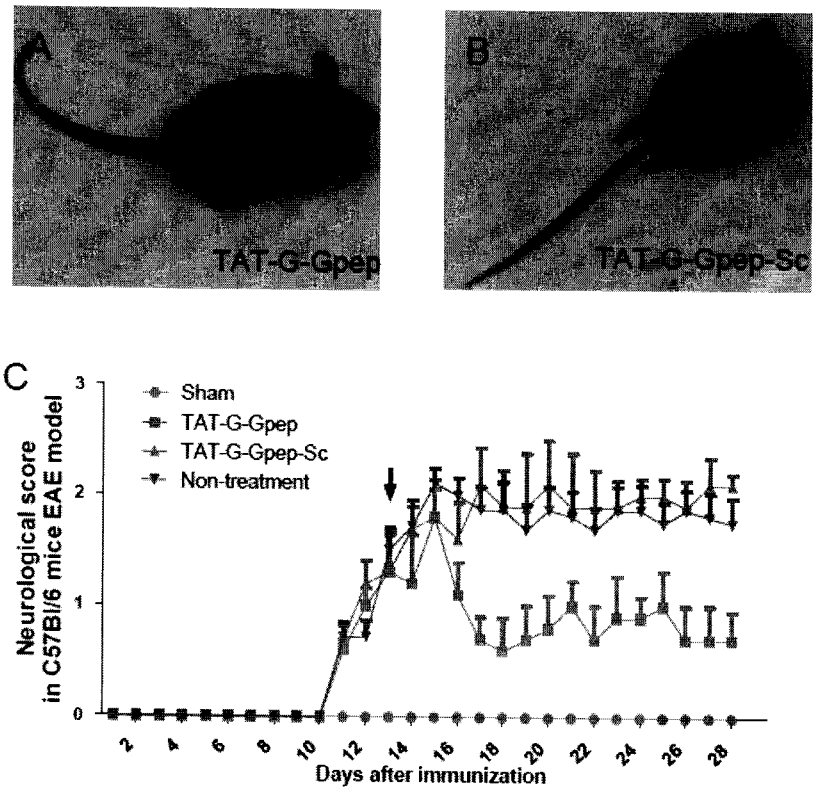
A
TAT-G-Gpep
B
TAT-G-Gpep-Sc
C
Neurological score in C57Bl/6 mice EAE model
Sham
TAT-G-Gpep
TAT-G-Gpep-Sc
Non-treatment
0
1
2
3
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Days after immunization

FIG. 4

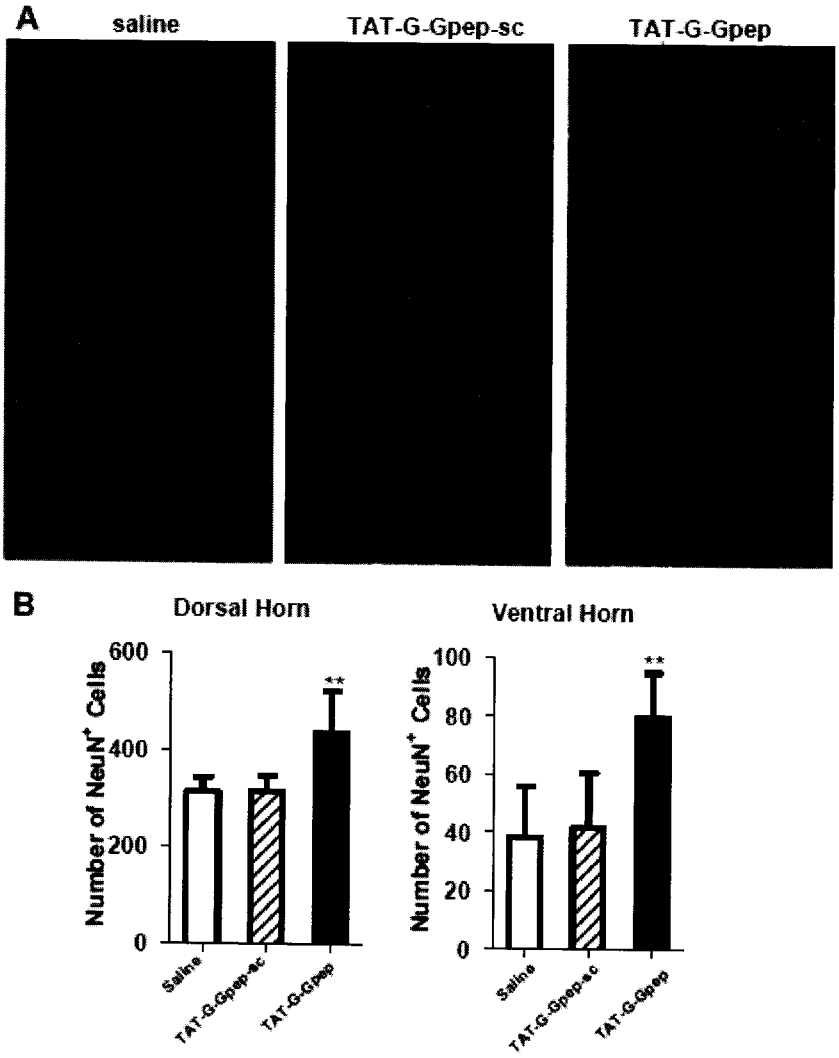
A
saline
TAT-G-Gpep-sc
TAT-G-Gpep
B
Dorsal Horn
Ventral Horn
Number of NeuN+ Cells
600
400
200
0
100
80
60
40
20
0
**
Saline
TAT-G-Gpep-sc
TAT-G-Gpep

FIG. 5

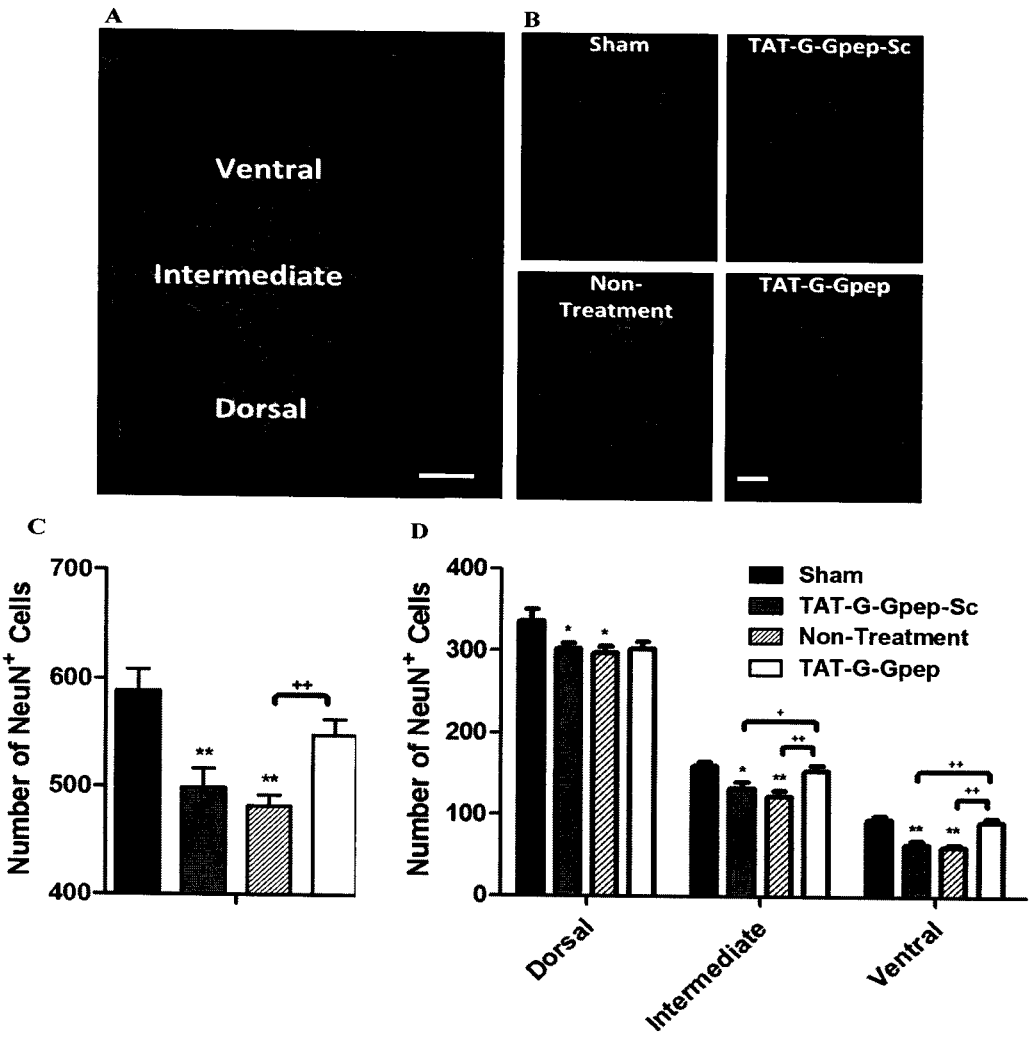
A
Ventral
Intermediate
Dorsal
B
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
C
Number of NeuN+ Cells
700
600
500
400
D
Number of NeuN+ Cells
400
300
200
100
0
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
Dorsal
Intermediate
Ventral

FIG. 6

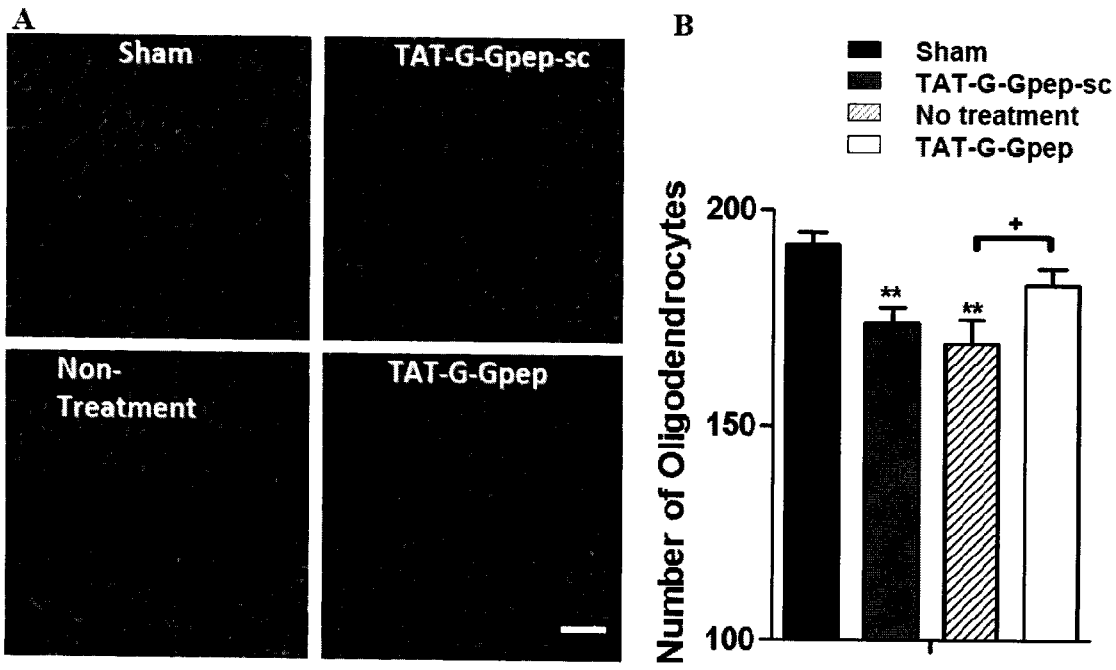
A
Sham
TAT-G-Gpep-sc
Non-
Treatment
TAT-G-Gpep
B
Sham
TAT-G-Gpep-sc
No treatment
TAT-G-Gpep
Number of Oligodendrocytes
200
150
100
**
**
+

FIG. 7

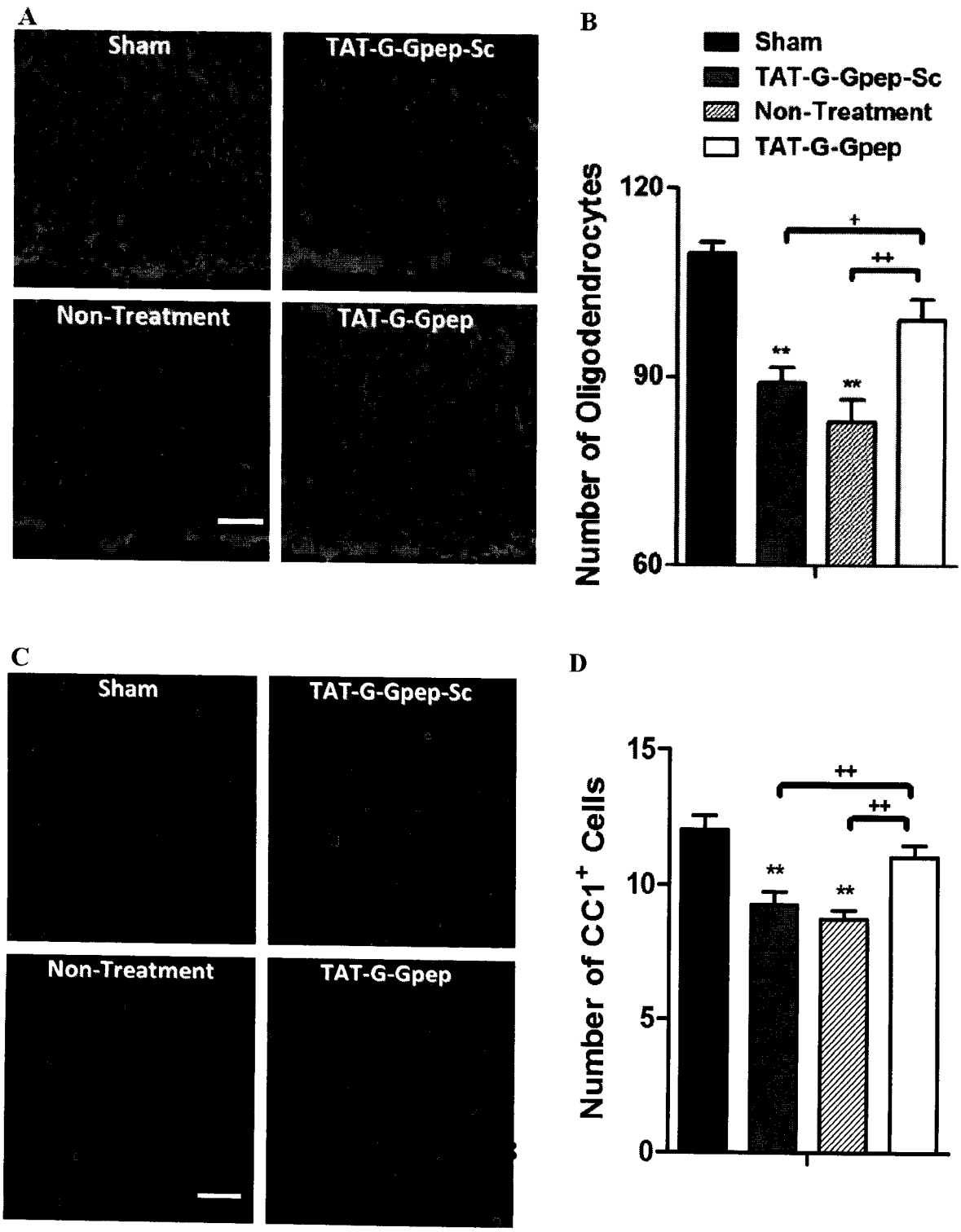
A
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
B
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
Number of Oligodendrocytes
120
90
60
+
++
**
**
C
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
D
Number of CC1+ Cells
15
10
5
0
++
++
**
**

FIG. 8.

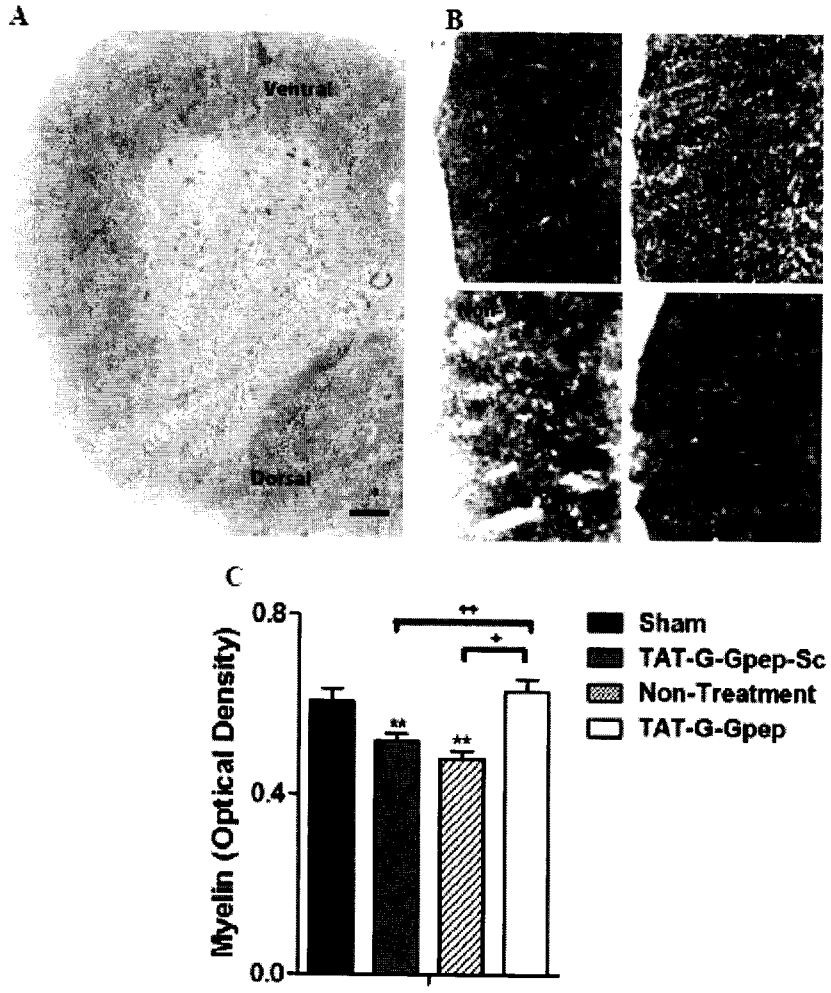
A
Ventral
Dorsal
B
C
Myelin (Optical Density)
0.8
0.4
0.0
**
**
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep

FIG. 9

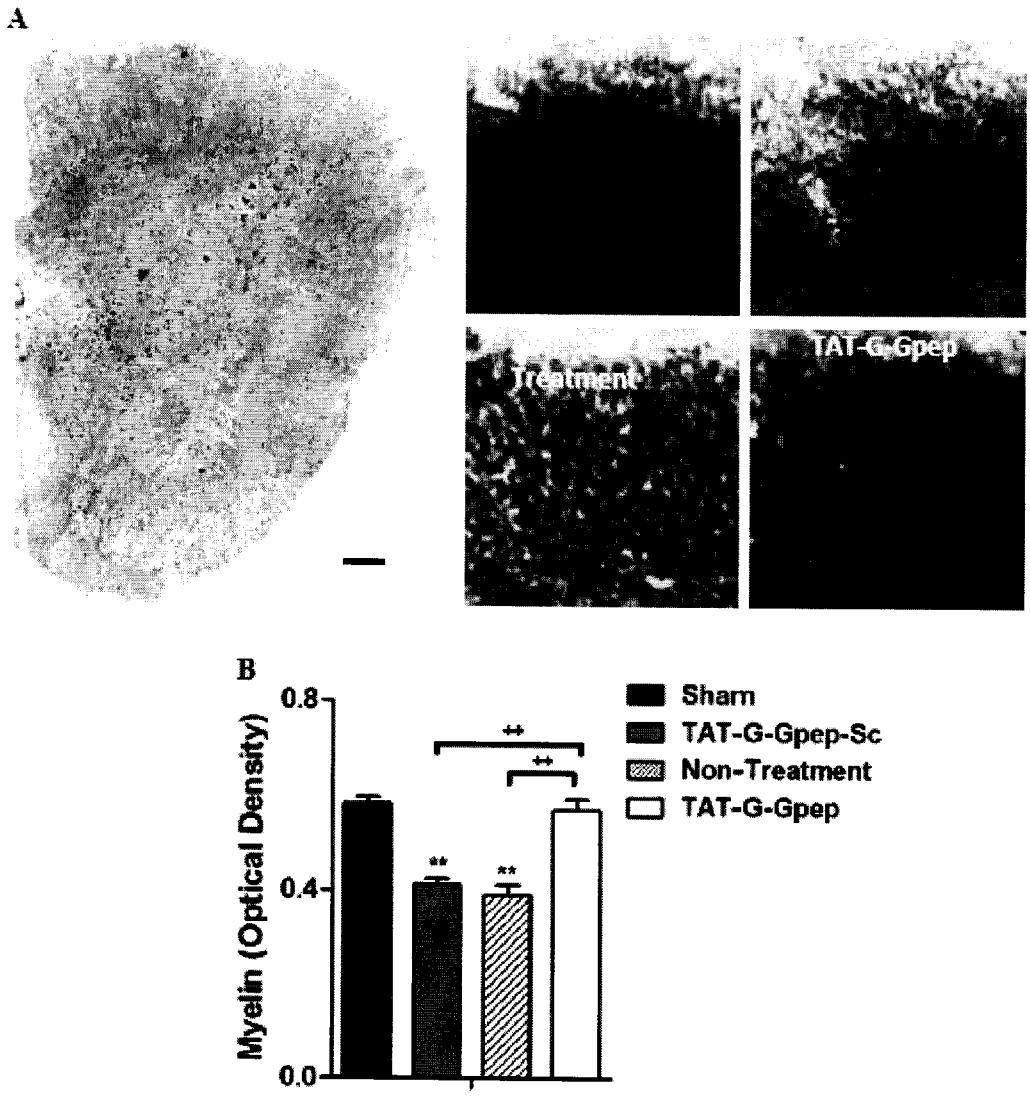
A
Treatment
TAT-G-Gpep
B
Myelin (Optical Density)
0.8
0.4
0.0
**
**
**
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep

FIG. 10

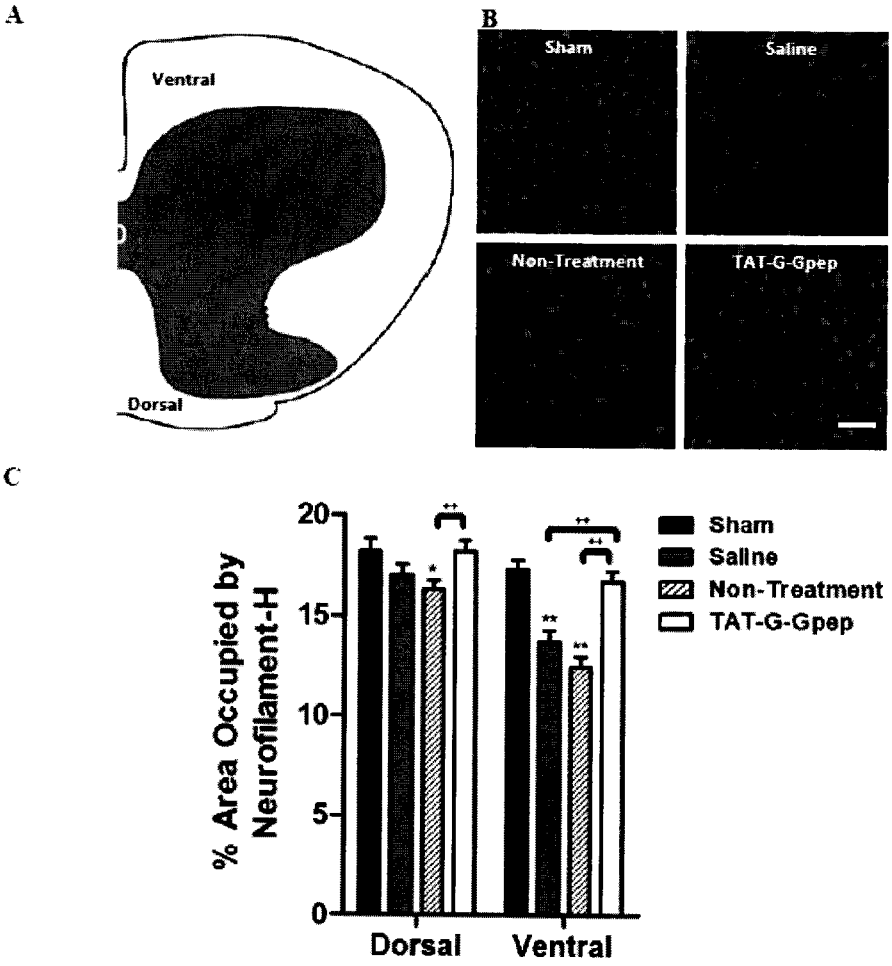
A
Ventral
Dorsal
B
Sham
Saline
Non-Treatment
TAT-G-Gpep
C
% Area Occupied by Neurofilament-H
20
15
10
5
0
Dorsal
Ventral
Sham
Saline
Non-Treatment
TAT-G-Gpep

FIG. 11

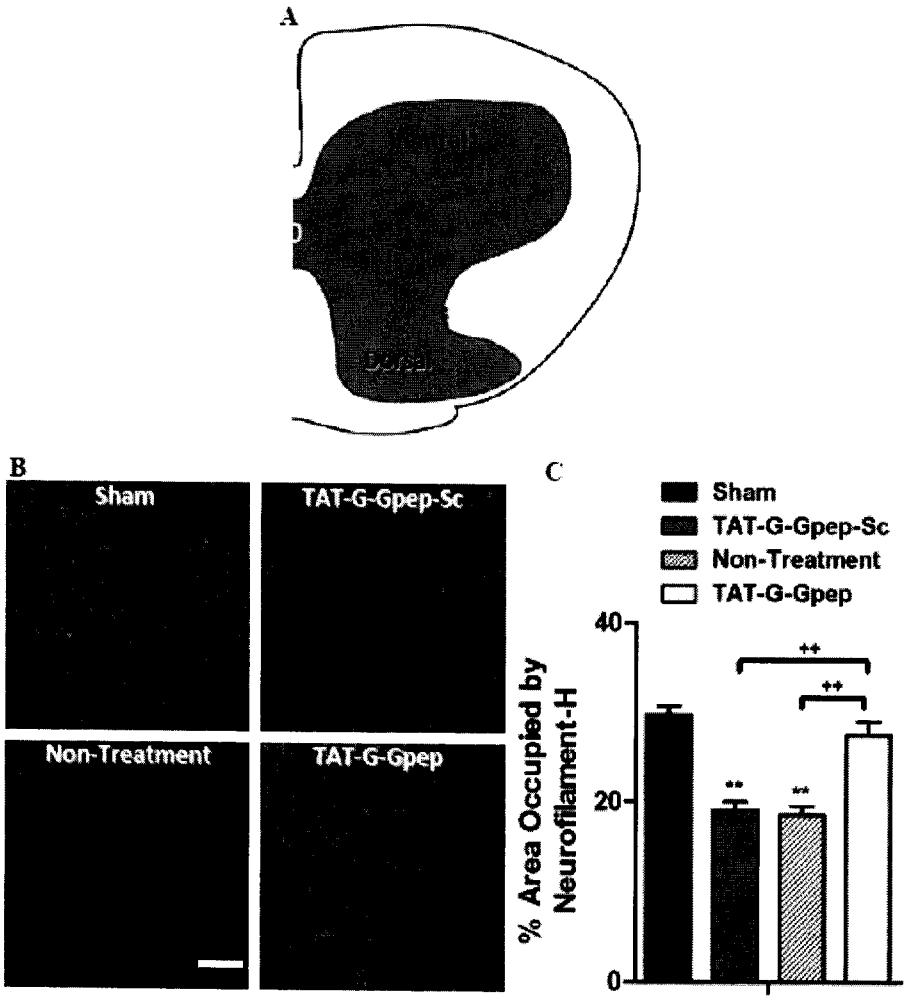
A
B
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
C
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
% Area Occupied by Neurofilament-H
40
20
0
++
++
**
**

FIG. 12

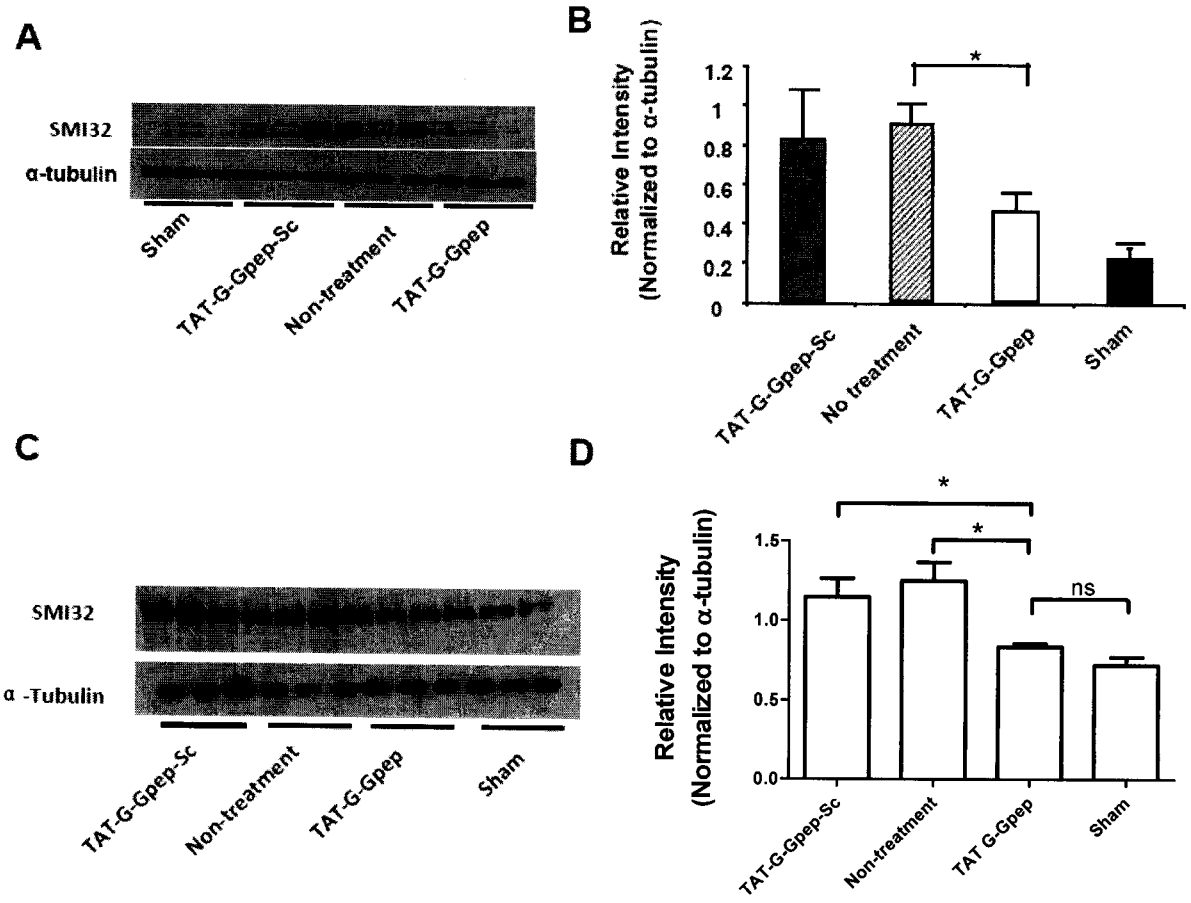
A
SMI32
α-tubulin
Sham
TAT-G-Gpep-Sc
Non-treatment
TAT-G-Gpep
B
Relative Intensity
(Normalized to α-tubulin)
1.2
1
0.8
0.6
0.4
0.2
0
*
TAT-G-Gpep-Sc
No treatment
TAT-G-Gpep
Sham
C
SMI32
α -Tubulin
TAT-G-Gpep-Sc
Non-treatment
TAT-G-Gpep
Sham
D
Relative Intensity
(Normalized to α-tubulin)
1.5
1.0
0.5
0.0
*
*
ns
TAT-G-Gpep-Sc
Non-treatment
TAT G-Gpep
Sham

FIG. 13

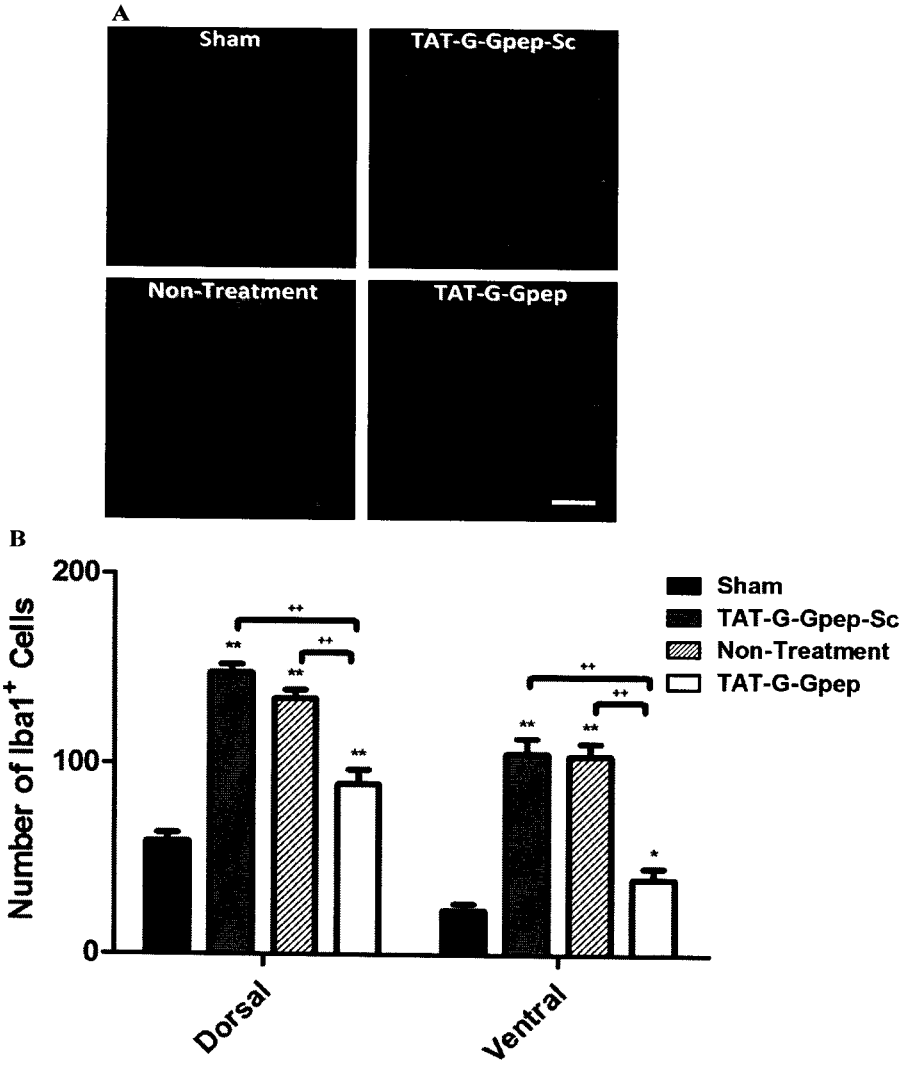
A
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep
B
Number of Iba1+ Cells
200
100
0
Dorsal
Ventral
Sham
TAT-G-Gpep-Sc
Non-Treatment
TAT-G-Gpep

FIG.14

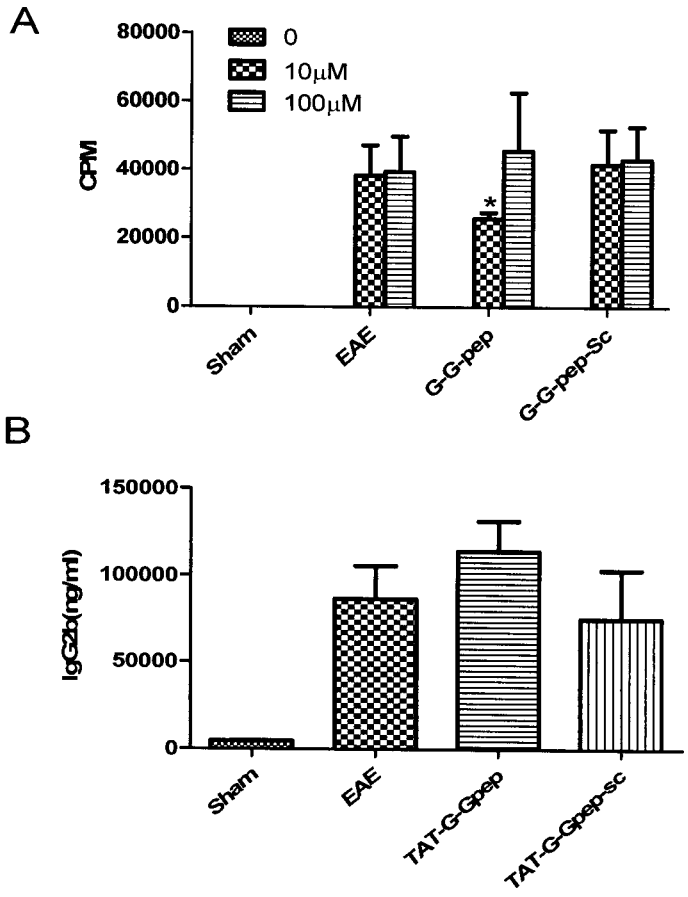
A
80000
60000
40000
20000
0
CPM
0
10μM
100μM
*
Sham
EAE
G-G-pep
G-G-pep-Sc
B
150000
100000
50000
0
IgG2b(ng/ml)
Sham
EAE
TAT-G-Gpep
TAT-G-Gpep-sc

FIG. 15

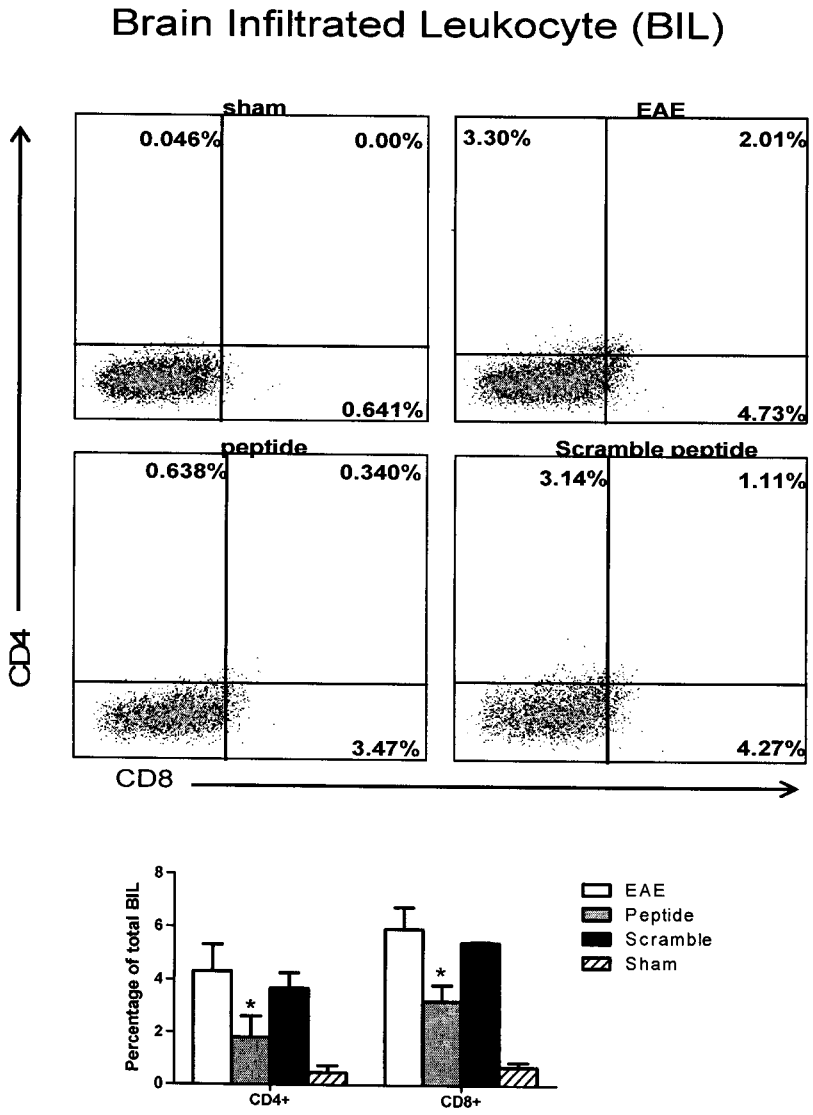
Brain Infiltrated Leukocyte (BIL)
sham
0.046%
0.00%
0.641%
EAE
3.30%
2.01%
4.73%
peptide
0.638%
0.340%
3.47%
Scramble peptide
3.14%
1.11%
4.27%
CD4
CD8
Percentage of total BIL
CD4+
CD8+
EAE
Peptide
Scramble
Sham

FIG. 16

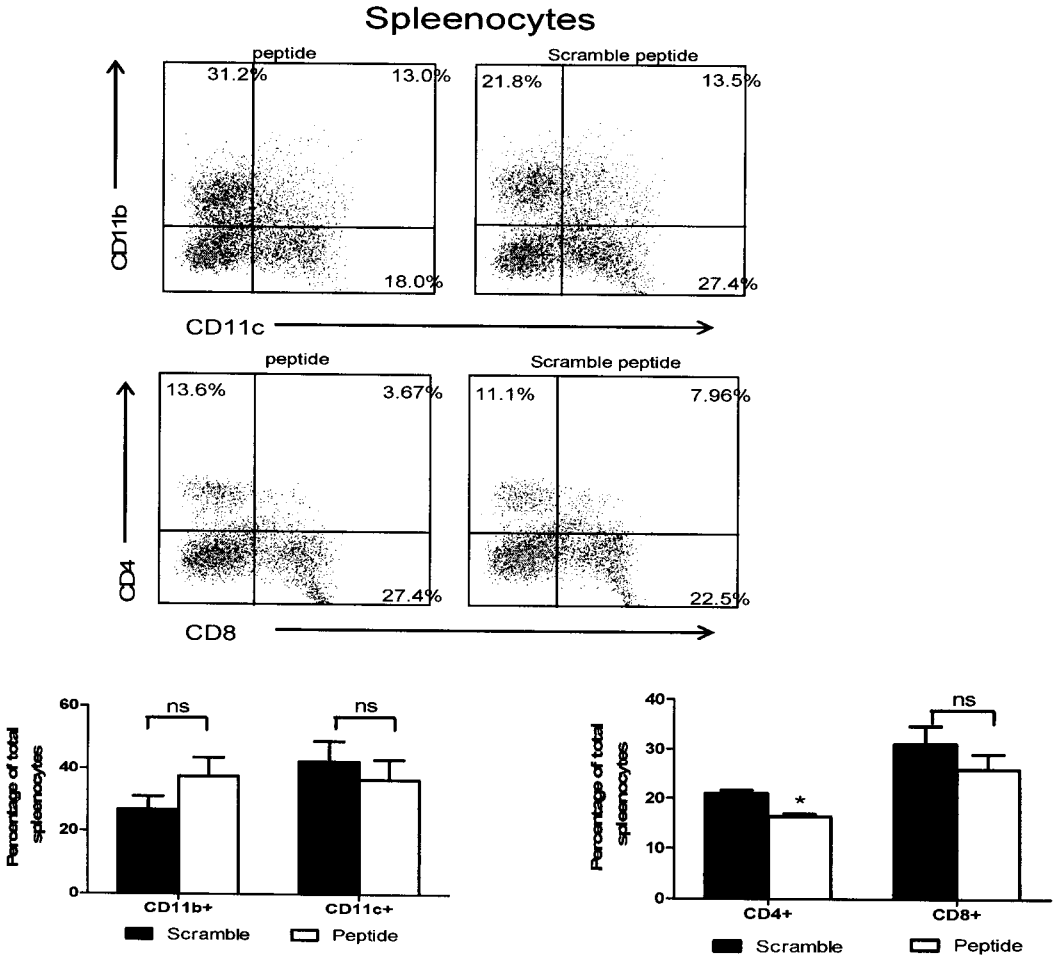
Spleenocytes
peptide
Scramble peptide
31.2%
13.0%
18.0%
21.8%
13.5%
27.4%
CD11b
CD11c
peptide
Scramble peptide
13.6%
3.67%
27.4%
11.1%
7.96%
22.5%
CD4
CD8
Percentage of total spleenocytes
ns
ns
CD11b+
CD11c+
Scramble
Peptide
*
CD4+
CD8+

FIG. 17

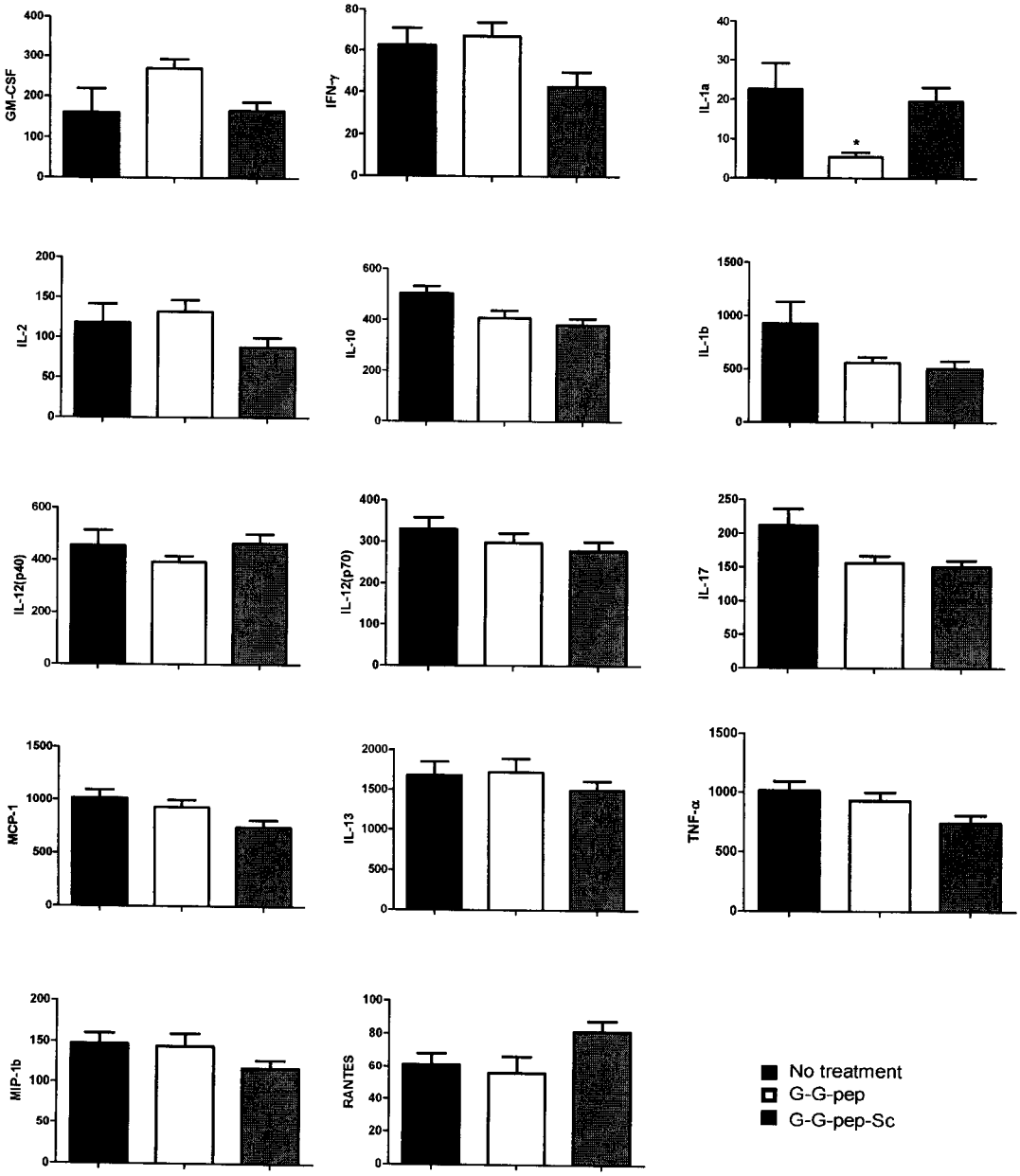
GM-CSF
IFN-γ
IL-1a
IL-2
IL-10
IL-1b
IL-12(p40)
IL-12(p70)
IL-17
MCP-1
IL-13
TNF-α
MIP-1b
RANTES
*
No treatment
G-G-pep
G-G-pep-Sc

FIG. 18

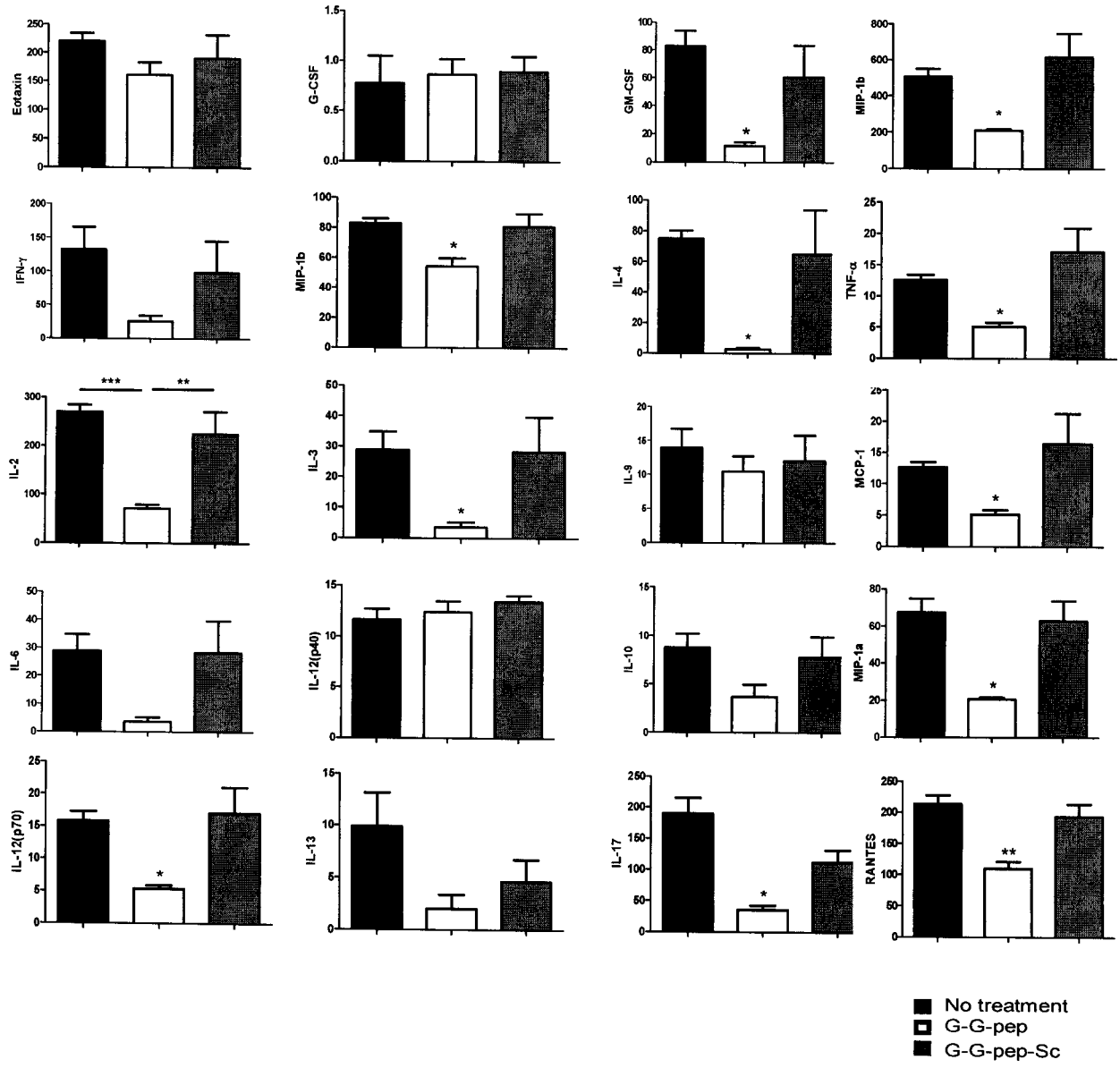
Eotaxin
G-CSF
GM-CSF
MIP-1b
IFN-γ
MIP-1b
IL-4
TNF-α
IL-2
IL-3
IL-9
MCP-1
IL-6
IL-12(p40)
IL-10
MIP-1a
IL-12(p70)
IL-13
IL-17
RANTES
No treatment
G-G-pep
G-G-pep-Sc

FIG. 19

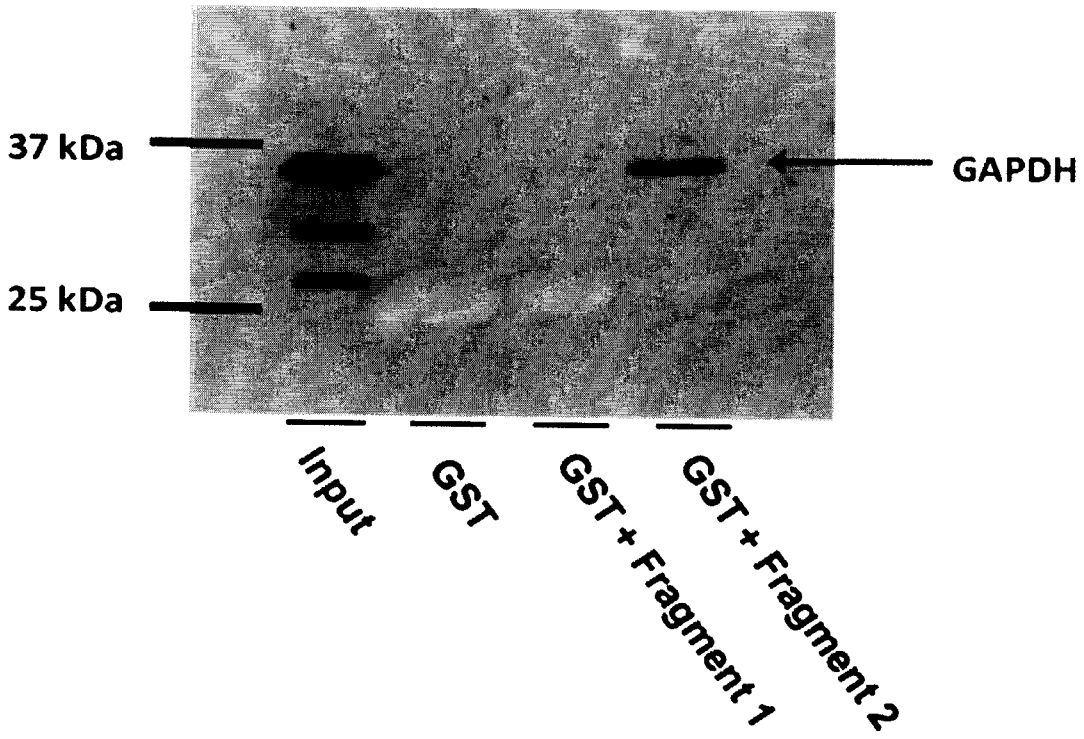

FIG. 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/000002

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 14/705*** (2006.01) , ***A61K 38/17*** (2006.01) , ***A61K 47/48*** (2006.01) , ***A61P 21/00*** (2006.01) , ***A61P 25/28*** (2006.01) , ***C07K 19/00*** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation seaixhed (classification system followed by classification symbols)

C07K 14/705 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

GenomeQuest, Scopus, TotalPatent

Keywords: SEQ ID Nos: 1 and 5

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO2008/025163 (LIU, F.) 6 March 2008 (06-03-2008)	

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"Y" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
March 15 2013	19 March 2013 (19-03-2013)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Ravinder Sardana (819) 997-4646

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/000002

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 7-22

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 7-22 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 7-22.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA20 13/000002

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2008025163A1	06 March 2008 (06-03-2008)	CA2662026A1	06 March 2008 (06-03-2008)
		EP2059598A1	20 May 2009 (20-05-2009)
		EP2059598A4	20 January 2010 (20-01-2010)
		US2010210521A1	19 August 2010 (19-08-2010)
		US81 19768B2	2 1 February 2012 (21-02-2012)
		US2012178696A1	12 July 2012 (12-07-2012)

C07K 7/08 **(2006.01)** **.** ***C12N 15/12*** **(2006.01)**