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(54) Benævnelse: **AAV-BASED GENE THERAPY FOR MULTIPLE SCLEROSIS**

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DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 61/983,924, filed April 24, 2014.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates generally to the fields of molecular biology and virology, and in particular, to the development of gene therapy vectors and methods for treatment of autoimmune diseases, such as multiple sclerosis (MS).

BACKGROUND

[0003] ***Multiple Sclerosis (MS).*** MS is a multifocal demyelinating disease with progressive neurodegeneration caused by an autoimmune response to self-antigens in a genetically susceptible individual. Depending on where in the CNS the damage occurs, symptoms may include problems with muscle control, balance, vision, or speech. 250,000 to 350,000 people in the US. MS is an autoimmune disease that develops (in part) from a failure of central and peripheral tolerance mechanisms (particularly Tregs) to maintain self-tolerance and control potentially pathogenic autoreactive lymphocytes.[2,3] It is characterized by chronic lymphocyte infiltration and inflammation of the CNS resulting in demyelination.

[0004] ***Gene Therapy.*** Major advances in the field of gene therapy have been achieved by using viruses to deliver therapeutic genetic material. The adeno-associated virus (AAV) has attracted considerable attention as a highly effective viral vector for gene therapy due to its low immunogenicity and ability to effectively transduce non-dividing cells. AAV has been shown to infect a variety of cell and tissue types, and significant progress has been made over the last decade to adapt this viral system for use in human gene therapy.

[0005] In its normal "wild type" form, AAV DNA is packaged into the viral capsid as a single-stranded molecule about 4600 nucleotides (nt) in length. Following infection of the cell by the virus, the molecular machinery of the cell converts the single-stranded DNA into a double-stranded form. Only this double-stranded DNA form can be transcribed by cellular enzymes into RNA, which is then translated into polypeptides by additional cellular pathways.

BRIEF SUMMARY OF THE DISCLOSURE

[0006] What is specifically lacking in the prior art, however, are viral vector-based gene therapy methods for treating and/or ameliorating one or more of symptoms of autoimmune diseases, including, for example, MS. The development of such vectors, and compositions comprising them would provide a major advancement in medicine, and particularly in the development of a gene therapy-based treatment modality for MS. The present invention is defined in the accompanying claims and relates to recombinant adeno-associated viral (rAAV) nucleic acid vectors, rAAV particles, rAAV vectors and rAAV particles for use in medicine, and rAAV vectors and rAAV particles for use in the treatment or amelioration of one or more symptoms of an autoimmune disease in a mammal. Embodiments of the invention are described in the following numbered paragraphs:

1. (1). A recombinant adeno-associated viral (rAAV) nucleic acid vector of the AAV8 serotype comprising:
   a polynucleotide that includes a nucleic acid segment that encodes a full-length mammalian myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) operably linked to a promoter that is capable of expressing the nucleic acid segment in one or more cells of a mammalian liver.
2. (2). The rAAV nucleic acid vector of paragraph 1, wherein the MBP, PLP or MOG are of human origin.
3. (3). The rAAV nucleic acid vector of paragraph 1, wherein the nucleic acid segment encodes a myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) that comprises an amino acid sequence that is at least 95% identical to the sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.
4. (4). The rAAV nucleic acid vector of any one of paragraphs 1 to 3, wherein the nucleic acid segment further comprises an enhancer, a post-transcriptional regulatory sequence, a polyadenylation signal, or any combination thereof, operably linked to the nucleic acid segment encoding the therapeutic molecule.
5. (5). The rAAV nucleic acid vector of any one of paragraphs 1 to 4, wherein the promoter is a mammalian cell-specific or a mammalian tissue-specific promoter.
6. (6). The rAAV nucleic acid vector of any one of paragraphs 1 to 5, wherein the nucleic acid segment further encodes or further expresses a polypeptide, a peptide, a ribozyme, a peptide nucleic acid, an siRNA, an RNAi, an antisense oligonucleotide, an antisense polynucleotide, an antibody, an antigen binding fragment, or any combination thereof.
7. (7). A rAAV particle comprising the rAAV nucleic acid vector of any one of paragraphs 1 to 6.
8. (8). The rAAV nucleic acid vector of any one of paragraphs 1 to 6 or the rAAV particle of paragraph 7 for use in medicine.
9. (9). The rAAV nucleic acid vector of any one of paragraphs 1 to 6 or the rAAV particle of paragraph 7 for use in the treatment or amelioration of one or more symptoms of an autoimmune disease in a mammal, wherein the rAAV nucleic acid vector or rAAV particle are systemically administered to the mammal, in an amount and for a time sufficient to treat or ameliorate the one or more symptoms of the autoimmune disease in the mammal, wherein the autoimmune disease is multiple sclerosis, disseminated sclerosis, or encephalomyelitis disseminata.

10. (10). The rAAV nucleic acid vector or rAAV particle for use according to paragraph 9, wherein the mammal is a newborn, an infant, a juvenile, or a young adult.
11. (11). The rAAV nucleic acid vector or rAAV particle for use according to paragraph 9 or paragraph 10, wherein production of the therapeutic molecule in the mammal reduces CNS inflammation, inhibits demyelination, re-establishes immune tolerance to one or more neuroproteins, stimulates the production of endogenous antigen-specific regulatory T cells, or any combination thereof.
12. (12). The rAAV nucleic acid vector or rAAV particle for use according to any one of paragraphs 9 to 11, wherein the rAAV vector further encodes an agonist, an antagonist, an anti-apoptosis factor, an inhibitor, a receptor, a cytokine, a cytotoxin, an erythropoietic agent, a glycoprotein, a growth factor, a growth factor receptor, a hormone, a hormone receptor, an interferon, an interleukin, an interleukin receptor, a nerve growth factor, a neuroactive peptide, a neuroactive peptide receptor, a protease, a protease inhibitor, a protein decarboxylase, a protein kinase, a protein kinase inhibitor, an enzyme, a receptor binding protein, a transport protein or an inhibitor thereof, a serotonin receptor, or an uptake inhibitor thereof, a serpin, a serpin receptor, a tumour suppressor, a chemotherapeutic, or any combination thereof.
13. (13). The rAAV nucleic acid vector of paragraph 2, wherein the nucleic acid segment encodes a myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) that comprises an amino acid sequence that is at least 95% identical to the sequence of SEQ ID NO:17, SEQ ID NO:29, or SEQ ID NO:15.
14. (14). The rAAV nucleic acid vector of paragraph 2, wherein the nucleic acid segment encodes a myelin basic protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG) that comprises an amino acid sequence as set forth in SEQ ID NO:17, SEQ ID NO:29, or SEQ ID NO:15.

[0007] The present disclosure overcomes these and other limitations inherent in the prior art by providing, in part, novel AAV nucleic acid vectors that are capable of, and optimized for, liver-directed expression of full-length proteins such as neuroproteins (including, without limitation, MOG, PLP, and MBP) or functional fragments thereof, thus abrogating the need for identifying HLA-MHC-specific epitopes required for inducing antigen-specific Tregs. In some options, the disclosure permits each patient undergoing treatment to generate his/her own unique antigen-specific Tregs, which makes the treatment more universally applicable and more clinically feasible than existing technologies.

[0008] 2 million people worldwide are living with MS. Diagnosis generally occurs at ages of 20 to 40, but documented cases of MS in children as young as two have been reported. Schilder's disease is a rare progressive demyelinating disorder that usually begins in childhood. While there is currently no cure for MS, there are various MS treatment options which have shown a decrease in the severity and frequency of relapses and a delay in disease progression in numerous studies. Therapies with predominantly immunomodulating properties include e.g., Beta-interferons and Glatiramer acetate. Therapies with predominantly immunosuppressive

properties include e.g., Natalizumab, Fingolimod, and Mitoxantrone. The development of protocols that stimulates Treg numbers and/or their function has become a significant focus in treating autoimmune diseases. In fact, many of the beneficial effects of currently FDA approved immune-modulators used in the treatment of MS are associated with restored Treg homeostasis.[2,4,5]

[0009] AAV gene therapy has been proven to be a powerful new tool for the treatment of a broad spectrum of diseases, including restoration of vision in patients with Leber's congenital amaurosis by retinal gene transfer, and hemophilia B by hepatic gene therapy.[6,7] According to aspects of the disclosure, it has been demonstrated that hepatic gene therapy transfer with AAV vectors can reliably induce a robust antigen-specific immune tolerance in experimental animals to a variety of proteins even when the antigen is subsequently expressed in a highly immunogenic manner in other organs. Together these results demonstrate that liver directed gene therapy can abrogate potential cytotoxic $CD8^+$ T cell responses.[1,8-13] Importantly, it has also been shown that this protocol can even eliminate pre-existing antibodies.[1] This finding is quite significant since there is an increasing body of evidence that B cells and autoantibodies may play a pathogenic role in demyelinating disease.[14,15] Others have shown that transgenic mice or transient transfection by plasmid or Ad-vectors expressing myelin basic protein could prevent the onset of EAE disease in mice.[16,17] Suppression was dependent on hepatic gene expression and was mediated by induction of Ag-specific Tregs. In contrast, aspects of the disclosure relate to treatment of certain autoimmune conditions, e.g., MS, utilizing AAV delivery of nucleic acids encoding one or more host proteins to the liver.

[0010] Hepatocyte-restricted transgene expression from an optimized AAV vector can reliably induce immune tolerance to various therapeutic proteins (e.g., mediated by Ag-specific $CD4^+CD25^+FoxP3^+$ Tregs). The process suppresses antibody formation and cytotoxic $CD8^+$ T cell responses against the transgene product. Hepatic transgene expression is maintained even when the antigen was subsequently expressed in a highly immunogenic manner in other organs. The process efficiently and rapidly reverses pre-existing high antibody titers, and provided long-term correction of haemostasis in a murine hemophilia B model. Importantly, the method does not require protein to be secreted to be functional.

[0011] In some options, advantageously, the novel rAAV nucleic acid vectors, express constructs, and infectious virions and viral particles comprising them as disclosed herein preferably have an improved efficiency in transducing one or more mammlalian liver cells to provide persistent expression of one or more genes of interest.

[0012] The improved rAAV nucleic acid vectors provided hererin preferably transduce mammalian cells with sufficient transduction efficiency to suppress the immune response associated with MS in patients, and thus abrogate CNS inflammation, and immune-mediated damage that occurs in MS patients. Unlike current therapies, this gene-therapy based approach represents a persistent, long-term treatment that reduces the clinical disability

experienced by MS patients.

[0013] In some options, the disclosure provides improved rAAV particles including those derived from one or more serotypes as known in the art (including, for example, those selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12).

[0014] The present disclosure also concerns rAAV nucleic acid vectors, wherein the nucleic acid segment further comprises a promoter, an enhancer, a post-transcriptional regulatory sequence, a polyadenylation signal, or any combination thereof, operably linked to the nucleic acid segment that encodes the selected polynucleotide of interest.

[0015] In certain options, the nucleic acid segments cloned into the novel rAAV expression vectors described herein will express or encode one or more polypeptides, peptides, ribozymes, peptide nucleic acids, siRNA's, RNAi's, antisense oligonucleotides, antisense polynucleotides, antibodies, antigen binding fragments, or any combination thereof.

[0016] As noted herein, the therapeutic agents useful in the disclosure may include one or more agonists, antagonists, anti-apoptosis factors, inhibitors, receptors, cytokines, cytotoxins, erythropoietic agents, glycoproteins, growth factors, growth factor receptors, hormones, hormone receptors, interferons, interleukins, interleukin receptors, nerve growth factors, neuroactive peptides, neuroactive peptide receptors, proteases, protease inhibitors, protein decarboxylases, protein kinases, protein kinase inhibitors, enzymes, receptor binding proteins, transport proteins or one or more inhibitors thereof, serotonin receptors, or one or more uptake inhibitors thereof, serpins, serpin receptors, tumor suppressors, diagnostic molecules, chemotherapeutic agents, cytotoxins, or any combination thereof.

[0017] The rAAV nucleic acid vectors of the present disclosure may be comprised within a virion or viral particle having a serotype that is selected from the group consisting of AAV serotype 1 (AAV1), AAV serotype 2 (AAV2), AAV serotype 3 (AAV3), AAV serotype 4 (AAV4), AAV serotype 5 (AAV5), AAV serotype 6 (AAV6), AAV serotype 7 (AAV7), AAV serotype 8 (AAV8), AAV serotype 9 (AAV9), AAV serotype 10 (AAV10), AAV serotype 11 (AAV11), or AAV serotype 12 (AAV12), or any other serotype as known to one of ordinary skill in the viral arts.

[0018] In related options, the disclosure further provides populations and pluralities of rAAV nucleic acid vectors, virions, infectious viral particles, or host cells that include one or more nucleic acid segments that encode an autoimmune disease therapeutic agent.

[0019] The disclosure further provides compositions and formulations that include one or more of the proteins, nucleic acid segments, viral vectors, host cells, or viral particles of the present disclosure together with one or more pharmaceutically-acceptable buffers, diluents, or excipients. Such compositions may be included in one or more diagnostic or therapeutic kits, for diagnosing, preventing, treating or ameliorating one or more symptoms of a mammalian inflammatory disease, such as autoimmune disease, and in particular, for delivery of a

therapeutic agent for the treatment of MS in a human.

[0020] The disclosure further includes a method for providing a mammal in need thereof with a diagnostically- or therapeutically-effective amount of a selected therapeutic agent, the method comprising administering to a cell, tissue or organ of a mammal in need thereof, an amount of one or more of the disclosed rAAV nucleic acid vectors; and for a time effective to provide the mammal with a diagnostically- or a therapeutically-effective amount of the selected therapeutic agent.

[0021] The disclosure further provides a method for diagnosing, preventing, treating, or ameliorating at least one or more symptoms of a disease, a disorder, a dysfunction, an injury, an abnormal condition, or trauma in a mammal. In an overall and general sense, the method includes at least the step of administering to a mammal in need thereof one or more of the disclosed rAAV nucleic acid vectors, in an amount and for a time sufficient to diagnose, prevent, treat or ameliorate the one or more symptoms of the disease, disorder, dysfunction, injury, abnormal condition, or trauma in the mammal.

[0022] The disclosure also provides a method of transducing a population of mammalian cells. In an overall and general sense, the method includes at least the step of introducing into one or more cells of the population, a composition that comprises an effective amount of one or more of the rAAV nucleic acid vectors disclosed herein.

[0023] In some options, the disclosure also provides isolated nucleic acid segments that encode one or more of the rAAV vector-based gene therapy constructs as described herein, and provides recombinant vectors, virus particles, infectious virions, and isolated host cells that comprise one or more of the rAAV nucleic acid vectors described herein.

[0024] Additionally, the present disclosure provides compositions, as well as therapeutic and/or diagnostic kits that include one or more of the disclosed AAV nucleic acid vector or AAV particle compositions, formulated with one or more additional ingredients, or prepared with one or more instructions for their use.

[0025] In one aspect, the disclosure provides compositions comprising recombinant adeno-associated viral (AAV) nucleic acid vectors, virions, viral particles, and pharmaceutical formulations thereof, useful in methods for delivering genetic material encoding one or more beneficial or therapeutic product(s) to mammalian cells and tissues. In some options, the compositions and methods of the disclosure provide a significant advancement in the art through their use in the treatment, prevention, and/or amelioration of symptoms of one or more mammalian inflammatory diseases, including autoimmune diseases such as MS and the like.

[0026] In some options, the disclosure provides rAAV-based expression constructs that encode one or more mammalian therapeutic agent(s) (including, but not limited to, for example, protein(s), polypeptide(s), peptide(s), enzyme(s), antibodies, antigen binding fragments, as well as variants, and/or active fragments thereof), for use in the treatment, prophylaxis, and/or

amelioration of one or more symptoms of a mammalian disease, dysfunction, injury, and/or disorder.

[0027] The improved nucleic acid vectors and expression systems of the present disclosure may also optionally further include a polynucleotide that comprises, consists essentially of, or consists of, one or more polylinkers, restriction sites, and/or multiple cloning region(s) to facilitate insertion (cloning) of one or more selected genetic elements, genes of interest, or therapeutic or diagnostic constructs into the rAAV vector at a selected site within the vector.

[0028] In further aspects of the present disclosure, the exogenous polynucleotide(s) that may be delivered into suitable host cells by the rAAV nucleic acid vectors disclosed herein are preferably of mammalian origin, with polynucleotides encoding one or more polypeptides or peptides of human, non-human primate, porcine, bovine, ovine, feline, canine, equine, epine, caprine, or lupine origin being particularly preferred.

[0029] The exogenous polynucleotide(s) that may be delivered into host cells by the disclosed viral nucleic acid vectors may, in certain options, encode one or more proteins, one or more polypeptides, one or more peptides, one or more enzymes, or one or more antibodies (or antigen-binding fragments thereof), or alternatively, may express one or more siRNAs, ribozymes, antisense oligonucleotides, PNA molecules, or any combination thereof. When combinational gene therapies are desired, two or more different molecules may be produced from a single rAAV expression system, or alternatively, a selected host cell may be transfected with two or more unique rAAV expression systems, each of which may comprise one or more distinct polynucleotides that encode a therapeutic agent.

[0030] In other options, the disclosure also provides rAAV nucleic acid vectors that are comprised within an infectious adeno-associated viral particle or a virion, as well as pluralities of such virions or infectious particles. Such vectors, particles, and virions may be comprised within one or more diluents, buffers, physiological solutions or pharmaceutical vehicles, or formulated for administration to a mammal in one or more diagnostic, therapeutic, and/or prophylactic regimens. The vectors, virus particles, virions, and pluralities thereof of the present disclosure may also be provided in excipient formulations that are acceptable for veterinary administration to selected livestock, exotics, domesticated animals, and companion animals (including pets and such like), as well as to non-human primates, zoological or otherwise captive specimens, and such like.

[0031] The disclosure also concerns host cells that comprise at least one of the disclosed rAAV nucleic acid expression vectors, or one or more virus particles or virions that comprise such an expression vector. Such host cells are particularly mammalian host cells, with human liver cells being particularly highly preferred, and may be either isolated, in cell or tissue culture. In the case of genetically modified animal models, the transformed host cells may even be comprised within the body of a non-human animal itself.

[0032] Compositions comprising one or more of the disclosed rAAV nucleic acid vectors,

expression systems, infectious rAAV particles, or host cells also form part of the present disclosure, and particularly those compositions that further comprise at least a first pharmaceutically-acceptable excipient for use in therapy, and for use in the manufacture of medicaments for the treatment of one or more mammalian inflammatory diseases, disorders, dysfunctions, or trauma. Such pharmaceutical compositions may optionally further comprise one or more diluents, buffers, liposomes, a lipid, a lipid complex. Alternatively, the rAAV nucleic acid vectors or rAAV particles of the present disclosure may be comprised within a plurality of microspheres, nanoparticles, liposomes, or any combination thereof.

[0033] Kits comprising one or more of the disclosed rAAV nucleic acid vectors (as well as one or more virions, viral particles, transformed host cells or pharmaceutical compositions comprising such vectors, virons, particle, or host cells); and instructions for using such kits in one or more therapeutic, diagnostic, and/or prophylactic clinical options are also provided by the present disclosure. Such kits may further comprise one or more reagents, restriction enzymes, peptides, therapeutics, pharmaceutical compounds, or means for delivery of the composition(s) to host cells, or to an animal (e.g., syringes, injectables, and the like). Exemplary kits include those for treating, preventing, or ameliorating the symptoms of a disease, deficiency, dysfunction, and/or injury, or may include components for the large-scale production of the viral vectors themselves, such as for commercial sale, or for use by others, including e.g., virologists, medical professionals, and the like.

[0034] Another important aspect of the present disclosure concerns methods of use of the disclosed rAAV nucleic acid vectors, virions, expression systems, compositions, and host cells described herein in the preparation of medicaments for diagnosing, preventing, treating or ameliorating at least one or more symptoms of a disease, a dysfunction, a disorder, an abnormal condition, a deficiency, injury, or trauma in an animal, and in particular, one or more autoimmune diseases in humans.

[0035] Compositions comprising one or more of the disclosed rAAV nucleic acid vectors, expression systems, infectious rAAV particles, and host cells also form part of the present disclosure, and particularly those compositions that further comprise at least a first pharmaceutically-acceptable excipient for use in the manufacture of medicaments and methods involving therapeutic administration of such rAAV nucleic vectors, rAAV particles, and host cells.

[0036] Another important aspect of the present disclosure concerns methods of use of the disclosed nucleic acid vectors, virions, expression systems, compositions, and host cells described herein in the preparation of medicaments for treating or ameliorating the symptoms of various autoimmune diseases, such as MS, in a mammal, and in particular one or more such diseases in a human.

[0037] In some options of any one of the method provided, the method further comprises administering an mTOR inhibitor, e.g., rapamycin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] For promoting an understanding of the principles of the disclosure, reference will now be made to the options, or examples, illustrated in the drawings and specific language will be used to describe the same. It will, nevertheless be understood that no limitation of the scope of the disclosure is thereby intended.

[0039] The following drawings form part of the present specification and are included to demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to the following description taken in conjunction with the accompanying drawings, in which like reference numerals identify like elements, and in which:

FIG. 1 describes aspects of an experimental autoimmune encephalomyelitis murine model employed in the present study as an animal model for MS;

FIG. 2A and FIG. 2B show a mouse model and mean clinical score criteria for the EAE study;

FIG. 3 shows a comparison of exemplary methods of the present disclosure as contrasted with the cell-based delivery methods of the prior art;

FIG. 4A and FIG. 4B show the AAV8 expression of MOG. FIG. 4A shows the Western blot analysis from protein extracted from liver, while FIG. 4B shows the analysis of transcriptional levels using real-time RT-PCR;

FIG. 5A shows the mean clinical score of EAE mice. Five female mice were injected *s.c.* with Ag/CFA emulsion. Mean clinical score (±SEM) was recorded starting at day 12;

FIG. 5B shows the mean clinical score of EAE mice. Five female C57BL/6 mice were injected s.c. with MOG/CFA emulsion. Mean clinical score (±SEM) was recorded;

FIG. 6A, FIG. 6B, and FIG. 6C show AAV8-MOG prevented development of EAE in C57BL/6 mice. C57BL/6 mice (n=5) were injected with AAV8-MOG or control. EAE was induced 2 weeks later. **FIG. 6A:** Mean clinical score, **FIG. 6B:** anti-MOG IgG1, **FIG. 6C:** anti-MOG IgG2c;

FIG. 7A, FIG. 7B, and FIG. 7C show that AAV8-vectored gene therapy prevents the onset of EAE in the animal model of MS;

FIG. 8 shows that AAV8-MOG ameliorated the disease in the animal model of MS;

FIG. 9 shows a PLP induced EAE naive control group to demonstrate disease progression;

FIG. 10 shows effective suppression of pre-existing disease using the AAV8-vectored MOG treatment;

FIG. 11 shows hepatic transgene expression of MOG. Western blot analysis from protein extracted from liver of MOG induced EAE mice injected with AAV8-MOG;

FIG. 12 shows Luxol Fast Blue (LFB) staining of spinal cords from mice that received AAV8-GFP and had EAE induced (A) or not (B);

FIG. 13A shows mean clinical score (MCS) in EAE-induced C57BL/6 mice that received AAV8-MOG or control vector after the mice reached a MCS of about 0.3. Bar graphs show statistical significance between final scores and peak-to-final scores;

FIG. 13B shows mean clinical score (MCS) in EAE-induced C57BL/6 mice that received AAV8-MOG or control vector after the mice reached a MCS of about 0.8. Bar graphs show statistical significance between final scores and peak-to-final scores;

FIG. 13C shows mean clinical score (MCS) in EAE-induced C57BL/6 mice that received AAV8-MOG or control vector after the mice reached a MCS of about 1.3. Bar graphs show statistical significance between final scores and peak-to-final scores;

FIG. 14A and 14B shows serial sections of spinal cord from an EAE induced female mouse ~35 days after receiving control vector (MCS=4.0). FIG. 14A is a hematoxylin and eosin stain showing areas of high inflammatory infiltration. FIG. 14B is a Luxol fast blue stain showing areas of demyelination. Circled areas highlight the co-localization of inflammation and loss of myelin;

FIG. 15A and 15B shows serial sections of spinal cord from an EAE induced female mouse ~35 days after receiving AAV-MOG vector. (MCS =1.25) FIG. 15A is a hematoxylin and eosin stain showing diminished infiltration. FIG. 15B is a Luxol fast blue stain which appears to show that the section has less areas of demyelination as a result of the suppression of the inflammation;

FIG. 16A and 16B show that Tregs isolated from spleens of AAV-MOG treated mice are functionally suppressive;

FIG. 17A, 17B, 17C and 17D show that AAV-MOG vector induces antigen specific Tregs. Splenocytes from mice injected with AAV-MOG vector 8 wks prior showed an increase in frequencies of I-Ab MOG_{35-55} Tetramer positive CD4+ (FIG. 17A) and Treg+ (FIG. 17C) compared to control tetramer positive CD+ (FIG. 17B) and Treg+ (FIG. 17D).

FIG. 18 shows that AAV8-PLP reduces clinical severity in mice with PLP induced relapsing-remitting EAE;

FIG. 19A shows aWestern blot analysis from protein extracted from liver of mice injected with AAV-MBP; and

FIG. 19B shows analysis of transcriptional levels of RNA obtained from the liver of mice treated with AAV-MBP or control by real-time RT-PCR.

DETAILED DESCRIPTION

[0040] Recombinant adeno-associated virus (AAV) vectors have been used successfully for *in vivo* gene transfer in numerous pre-clinical animal models of human disease, and have been used successfully for long-term expression of a wide variety of therapeutic genes (Daya and Berns, 2008; Niemeyer *et al.,* 2009; Owen *et al.,* 2002; Keen-Rhinehart *et al.,* 2005; Scallan *et al.,* 2003; Song *et al.,* 2004). AAV vectors have also generated long-term clinical benefit in humans when targeted to immune-privileged sites, e.g., ocular delivery for Leber's congenital amaurosis (Bainbridge *et al.,* 2008; Maguire *et al.,* 2008; Cideciyan *et al.,* 2008). A major advantage of this vector is its comparatively low immune profile, eliciting only limited inflammatory responses and, in some cases, even directing immune tolerance to transgene products (LoDuca *et al.,* 2009). Nonetheless, the therapeutic efficiency, when targeted to non-immune privileged organs, has been limited in humans due to antibody and $CD8^+$ T cell responses against the viral capsid, while in animal models, adaptive responses to the transgene product have also been reported (Manno *et al.,* 2006; Mingozzi *et al.,* 2007; Muruve *et al.,* 2008; Vandenberghe and Wilson, 2007; Mingozzi and High, 2007).

[0041] In some options, a rAAV nucleic acid vector described herein comprises inverted terminal repeat sequences (ITRs), such as those derived from a wild-type AAV genome, such as the AAV2 genome. In some options, the rAAV nucleic acid vector further comprises nucleic acid segment that includes a transgene (also referred to as a heterologous nucleic acid molecule) operably linked to a promoter and optionally, other regulatory elements, wherein the ITRs flank the nucleic acid segment.

[0042] In some options, the promoter is a mammalian cell-specific or a mammalian tissue-specific promoter. In some options, the promoter is a promoter that is capable of expressing the nucleic acid segment in one or more cells of a mammalian liver, such as hepatocyte cells. Exemplary hepatocyte specific promoters and enhancer elements include, e.g., albumin, human α1-antitrypsin (hAAT), transthyretin (TTR), and apolipoprotein E (apoE) promoters or enhancer elements.

[0043] In some options, the transgene encodes an autoimmune disease therapeutic molecule of interest, such as a mammalian myelin basis protein (MBP), proteolipid protein (PLP), or myelin oligodendrocyte glycoprotein (MOG). As used herein, an autoimmune therapeutic molecule includes any antigen (such as a protein, fragment thereof, or a peptide) that contributes to initiation and/or progression of an autoimmune disease. Exemplary autoimmune therapeutic molecules include myelin basis protein (MBP, e.g., for multiple sclerosis), proteolipid protein (PLP, e.g., for multiple sclerosis), myelin oligodendrocyte glycoprotein (MOG, e.g., for multiple sclerosis), myelin-associated glycoprotein (MAG, e.g., for Anti-MAG Peripheral Neuropathy), insulin (e.g., for type 1 diabetes), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP, e.g., for type 1 diabetes), Preproinsulin (e.g., for type 1 diabetes), Glutamic decarboxylase (GAD, e.g., for type 1 diabetes), tyrosine phosphatase like autoantigen (e.g., for type 1 diabetes), insulinoma antigen- 2 (e.g., for type 1 diabetes), Islet cell antigen (e.g., for type 1 diabetes); thyroid stimulating hormone (TSH) receptor (e.g., for

Graves disease), thyrotropin receptor (e.g., for Graves disease), Aggrecan (e.g., for rheumatoid arthritis), CD4+T cell epitope (GRVRVNSAY (SEQ ID NO: 36), e.g., for proteoglycaneinduced arthritis (PGIA) or rheumatoid arthritis), or acetylcholine receptor (e.g., for Myasthenia gravis). In some options, the autoimmune disease therapeutic molecule of interest is a human protein, such as human myelin basis protein (MBP), a human proteolipid protein (PLP), or a human myelin oligodendrocyte glycoprotein (MOG).

[0044] Exemplary transgene sequences (e.g., cDNA sequences) and protein sequences that may be encoded by the transgene are provided below. In some options, the transgene comprises a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any one of the cDNA sequences provided below (SEQ ID NOs: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34). In some options, the transgene comprises a sequence that is any one of the cDNA sequences provided below (SEQ ID NOs: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34). In some options, the transgene is codon-optimized for expression in human cells. In some options, the transgene contains a nucleotide sequence that encodes at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240 or more contiguous amino acids of any one of the protein sequences provided herein (e.g., any one of SEQ ID NOs: 1, 2, 3, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, or 35). In some options, the transgene contains a nucleotide sequence that encodes a protein that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any one of the protein sequences provided herein (e.g., any one of SEQ ID NOs: 1,2,3,9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, or 35). In some options, the transgene contains a nucleotide sequence that encodes any one of the protein sequences provided herein (e.g., any one of SEQ ID NOs: 1, 2, 3, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, or 35).

[0045] Exemplary Mus musculus myelin oligodendrocyte glycoprotein (MOG) cDNA

```
  1 atggcctgtt tgtggagctt ctctttgccc agctgcttcc tctcccttct cctcctcctt

 61 ctcctccagt tgtcatgcag ctatgcagga caattcagag tgataggacc agggtatccc
121 atccgggctt tagttgggga tgaagcagag ctgccgtgcc gcatctctcc tgggaaaaat
181 gccacgggca tggaggtggg ttggtaccgt tctcccttct caagagtggt tcacctctac
241 cgaaatggca aggaccaaga tgcagagcaa gcacctgaat accggggacg cacagagctt
301 ctgaaagaga ctatcagtga gggaaaggtt acccttagga ttcagaacgt gagattctca
361 gatgaaggag gctacacctg cttcttcaga gaccactctt accaagaaga ggcagcaatg
421 gagttgaaag tggaagatcc cttctattgg gtcaaccccg gtgtgctgac tctcatcgca
481 cttgtgccta cgatcctcct gcaggtctct gtaggccttg tattcctctt cctgcagcac
541 agactgagag gaaaacttcg tgcagaagta gagaatctcc atcggacttt tgatcctcac
601 ttcctgaggg tgccctgctg gaagataaca ctgtttgtta ttgtgcctgt tcttggaccc
661 ctggttgcct tgatcatctg ctacaactgg ctgcaccgaa gactggcagg acagtttctt
721 gaagagctaa gaaacccccct ttga (SEQ ID NO: 8)
```

[0046] Exemplary mus musculus myelin-oligodendrocyte glycoprotein (MOG) protein

```
  1 maclwsfswp scflslllll llqlscsyag qfrvigpgyp iralvgdeae lpcrispgkn
 61 atgmevgwyr spfsrvvhly rngkdqdaeq apeyrgrtel lketisegkv tlriqnvrfs
121 deggytcffr dhsyqeeaam elkvedpfyw vnpgvltlia lvptillqvs vglvflflqh
181 rlrgklraev enlhrtfdph flrvpcwkit lfvivpvlgp lvaliicynw lhrrlagqfl
241 eelrnpf (SEQ ID NO: 9)
```

[0047] Exemplary Mus musculus proteolipid protein 1 (PLP) cDNA

ATGGGCTTGTTAGAGTGTTGTGCTAGATGTCTGGTAGGGGCCCCCTTTGCTTCCCTGGTGGCCACTGGAT
TGTGTTTCTTTGGAGTGGCACTGTTCTGTGGATGTGGACATGAAGCTCTCACTGGTACAGAAAAGCTAAT
TGAGACCTATTTCTCCAAAAACTACCAGGACTATGAGTATCTCATTAATGTGATTCATGCTTTCCAGTAT
GTCATCTATGGAACTGCCTCTTTCTTCTTCCTTTATGGGGCCCTCCTGCTGGCTGAGGGCTTCTACACCA
CCGGCGCTGTCAGGCAGATCTTTGGCGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGCAACGGT
AACAGGGGGCCAGAAGGGGAGGGGTTCCAGAGGCCAACATCAAGCTCATTCTTTGGAGCGGGTGTGTCAT
TGTTTGGGAAAATGGCTAGGACATCCCGACAAGTTTGTGGGCATCACCTATGCCCTGACTGTTGTATGGC
TCCTGGTGTTTGCCTGCTCGGCTGTACCTGTGTACATTTACTTCAATACCTGGACCACCTGTCAGTCTAT
TGCCTTCCCTAGCAAGACCTCTGCCAGTATAGGCAGTCTCTGCGCTGATGCCAGAATGTATGGTGTTCTC
CCATGGAATGCTTTCCCTGGCAAGGTTTGTGGCTCCAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCC
AAATGACCTTCCACCTGTTTATTGCTGCGTTTGTGGGTGCTGCGGCCACACTAGTTTCCCTGCTCACCTT
CATGATTGCTGCCACTTACAACTTCGCCGTCCTTAAACTCATGGGCCGAGGCACCAAGTTCTGA (SEQ ID
NO: 10)

[0048] Exemplary Mus musculus proteolipid protein 1 (PLP) protein

```
  1 mglleccarc lvgapfaslv atglcffgva lfcgcgheal tqtekliety fsknyqdyey
 61 linvihafqy viygtasfff lygalllaeg fyttgavrqi fgdykticg kglsatvtgg
121 qkgrgsrgqh qahslervch clgkwlghpd kfvgityalt vvwllvfacs avpvyiyfnt
181 wttcqsiafp sktsasigsl cadarmygvl pwnafpgkvc gsnllsickt aefqmtfhlf
241 iaafvgaaat lvslltfmia atynfavlkl mgrgtkf (SEQ ID NO: 11)
```

[0049] Exemplary Mus musculus myelin basic protein (MBP) cDNA

ATGGGAAACCACTCTGGAAAGAGAGAATTATCTGCTGAGAAGGCCAGTAAGGATGGAGAGATTCACCGAG
GAGAGGCTGGAAAGAAGAGAAGCGTGGGCAAGCTTTCTCAGACGGCCTCAGAGGACAGTGATGTGTTTGG
GGAGGCAGATGCGATCCAGAACAATGGGACCTCGGCTGAGGACACGGCGGTGACAGACTCCAAGCACACA
GCAGACCCAAAGAATAACTGGCAAGGCGCCCACCCAGCTGACCCAGGGAACCGCCCCCACTTGATCCGCC
TCTTTTCCCGAGATGCCCCGGGAAGGGAGGACAACACCTTCAAAGACAGGCCCTCAGAGTCCGACGAGCT
TCAGACCATCCAAGAAGACCCCACAGCAGCTTCCGGAGGCCTGGATGTGATGGCATCACAGAAGAGACCC
TCACAGCGATCCAAGTACCTGGCCACAGCAAGTACCATGGACCATGCCAGGCATGGCTTCCTCCCAAGGC
ACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTAGCGGTGACAGGGGTGCGCCCAAGCGGGG
CTCTGGCAAGGTGAGCTCCGAGCCGTAG (SEQ ID NO: 12)

[0050] Exemplary Mus musculus myelin basic protein (MBP) protein

```
  1 mgnhsgkrel saekaskdge ihrgeagkkr svgklsqtas edsdvfgead aiqnngtsae
 61 dtavtdskht adpknnwqga hpadpgnrph lirlfsrdap gredntfkdr psesdelqti
121 qedptaasgg ldvmasqkrp sqrskylata stmdharhgf lprhrdtgil dsigrffsgd
181 rgapkrgsgk vssep (SEQ ID NO: 13)
```

[0051] Exemplary Homo sapiens myelin oligodendrocyte glycoprotein (MOG) cDNA

```
  1 atggcaagct tatcgagacc ctctctgccc agctgcctct gctccttcct cctcctcctc
 61 ctcctccaag tgtcttccag ctatgcaggg cagttcagag tgataggacc aagacaccct
121 atccgggctc tggtcgggga tgaagtggaa ttgccatgtc gcatatctcc tgggaagaac
181 gctacaggca tggaggtggg gtggtaccgc ccccccttct ctagggtggt tcatctctac
241 agaaatggca aggaccaaga tggagaccag gcacctgaat atcggggccg gacagagctg
301 ctgaaagatg ctattggtga gggaaaggtg actctcagga tccggaatgt aaggttctca
361 gatgaaggag gtttcacctg cttcttccga gatcattctt accaagagga ggcagcaatg
421 gaattgaaag tagaagatcc tttctactgg gtgagccctg gagtgctggt tctcctcgcg
481 gtgctgcctg tgctcctcct gcagatcact gttggcctcg tcttcctctg cctgcagtac
541 agactgagag gaaaacttcg agcagagata gagaatctcc accggacttt tgatccccac
601 tttctgaggg tgccctgctg gaagataacc ctgtttgtaa ttgtgccggt tcttggaccc
661 ttggttgcct tgatcatctg ctacaactgg ctacatcgaa gactagcagg gcaattcctt
721 gaagagctac gaaatccctt ctga (SEQ ID NO: 14)
```

[0052] Exemplary Homo sapiens myelin oligodendrocyte glycoprotein (MOG) protein

```
  1 maslsrpslp sclcsfllll llqvsssyag qfrvigprhp iralvgdeve lpcrispgkn
 61 atgmevgwyr ppfsrvvhly rngkdqdgdq apeyrgrtel lkdaigegkv tlrirnvrfs
```

```
121 deggftcffr dhsyqeeaam elkvedpfyw vspgvlvlla vlpvlllqit vglvflclqy
181 rlrgklraei enlhrtfdph flrvpcwkit lfvivpvlgp lvaliicynw lhrrlagqfl
241 eelrnpf (SEQ ID NO: 15)
```

[0053] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 7, cDNA

```
ATGGGAAACCACGCAGGCAAACGAGAATTAAATGCCGAGAAGGCCAGTACGAATAGTGAAACTAACAGAG
GAGAATCTGAAAAAAAGAGAAACCTGGGTGAACTTTCACGGACAACCTCAGAGGACAACGAAGTGTTCGG
AGAGGCAGATGCGAACCAGAACAATGGGACCTCCTCTCAGGACACAGCGGTGACTGACTCCAAGCGCACA
GCGGACCCGAAGAATGCCTGGCAGGATGCCCACCCAGCTGACCCAGGGAGCCGCCCCCACTTGATCCGCC
TCTTTTCCCGAGATGCCCCGGGGAGGGAGGACAACACCTTCAAAGACAGGCCCTCTGAGTCCGACGAGCT
CCAGACCATCCAAGAAGACAGTGCAGCCACCTCCGAGAGCCTGGATGTGATGGCGTCACAGAAGAGACCC
TCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACCATGCCAGGCATGGCTTCCTCC
CAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGGCGGTGACAGGGGTGCGCCCAA
GCGGGGCTCTGGCAAGGACTCACACCACCCGGCAAGAACTGCTCACTACGGCTCCCTGCCCCAGAAGTCA
CACGGCCGGACCCAAGATGAAAACCCCGTAGTCCACTTCTTCAAGAACATTGTGACGCCTCGCACACCAC
CCCCGTCGCAGGGAAAGGGGAGAGGACTGTCCCTGAGCAGATTTAGCTGGGGGGCCGAAGGCCAGAGACC
AGGATTTGGCTACGGAGGCAGAGCGTCCGACTATAAATCGGCTCACAAGGGATTCAAGGGAGTCGATGCC
CAGGGCACGCTTTCCAAAATTTTTAAGCTGGGAGGAAGAGATAGTCGCTCTGGATCACCCATGGCTAGAC
GCTGA (SEQ ID NO: 16)
```

[0054] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 7, protein

```
  1 mgnhagkrel naekastnse tnrgesekkr nlgelsrtts ednevfgead anqnngtssq
 61 dtavtdskrt adpknawqda hpadpgsrph lirlfsrdap gredntfkdr psesdelqti
121 qedsaatses ldvmasqkrp sqrhgskyla tastmdharh gflprhrdtg ildsigrffg
181 gdrgapkrgs gkdshhpart ahygslpqks hgrtqdenpv vhffknivtp rtpppsqgkg
241 rglslsrfsw gaegqrpgfg yggrasdyks ahkgfkgvda qgtlskifkl ggrdsrsgsp
301 marr (SEQ ID NO: 17)
```

[0055] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 1, cDNA

```
ATGGCGTCACAGAAGAGACCCTCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACC

ATGCCAGGCATGGCTTCCTCCCAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGG
CGGTGACAGGGGTGCGCCCAAGCGGGGCTCTGGCAAGGTACCCTGGCTAAAGCCGGGCCGGAGCCCTCTG
CCCTCTCATGCCCGCAGCCAGCCTGGGCTGTGCAACATGTACAAGGACTCACACCACCCGGCAAGAACTG
CTCACTACGGCTCCCTGCCCCAGAAGTCACACGGCCGGACCCAAGATGAAAACCCCGTAGTCCACTTCTT
CAAGAACATTGTGACGCCTCGCACACCACCCCCGTCGCAGGGAAAGGGGAGAGGACTGTCCCTGAGCAGA
TTTAGCTGGGGGGCCGAAGGCCAGAGACCAGGATTTGGCTACGGAGGCAGAGCGTCCGACTATAAATCGG
CTCACAAGGGATTCAAGGGAGTCGATGCCCAGGGCACGCTTTCCAAAATTTTTAAGCTGGGAGGAAGAGA
TAGTCGCTCTGGATCACCCATGGCTAGACGCTGA (SEQ ID NO: 18)
```

[0056] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 1, protein

```
  1 masqkrpsqr hgskylatas tmdharhgfl prhrdtgild sigrffggdr gapkrgsgkv
 61 pwlkpgrspl psharsqpgl cnmykdshhp artahygslp qkshgrtqde npvvhffkni
121 vtprtpppsq gkgrglslsr fswgaegqrp gfgyggrasd yksahkgfkg vdaqgtlski
181 fklggrdsrs gspmarr (SEQ ID NO: 19)
```

[0057] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 2, cDNA

```
ATGGCGTCACAGAAGAGACCCTCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACC
ATGCCAGGCATGGCTTCCTCCCAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGG
CGGTGACAGGGGTGCGCCCAAGCGGGGCTCTGGCAAGGTACCCTGGCTAAAGCCGGGCCGGAGCCCTCTG
CCCTCTCATGCCCGCAGCCAGCCTGGGCTGTGCAACATGTACAAGGACTCACACCACCCGGCAAGAACTG
CTCACTACGGCTCCCTGCCCCAGAAGTCACACGGCCGGACCCAAGATGAAAACCCCGTAGTCCACTTCTT
CAAGAACATTGTGACGCCTCGCACACCACCCCCGTCGCAGGGAAAGGGGGCCGAAGGCCAGAGACCAGGA
TTTGGCTACGGAGGCAGAGCGTCCGACTATAAATCGGCTCACAAGGGATTCAAGGGAGTCGATGCCCAGG
GCACGCTTTCCAAAATTTTTAAGCTGGGAGGAAGAGATAGTCGCTCTGGATCACCCATGGCTAGACGCTG
A (SEQ ID NO: 20)
```

[0058] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 2, protein

```
  1 masqkrpsqr hgskylatas tmdharhgfl prhrdtgild sigrffggdr gapkrgsgkv
 61 pwlkpgrspl psharsqpgl cnmykdshhp artahygslp qkshgrtqde npvvhffkni
121 vtprtpppsq gkgaegqrpg fgyggrasdy ksahkgfkgv daqgtlskif klggrdsrsg
181 spmarr (SEQ ID NO: 21)
```

[0059] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 3, cDNA

```
ATGGCGTCACAGAAGAGACCCTCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACC
ATGCCAGGCATGGCTTCCTCCCAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGG
CGGTGACAGGGGTGCGCCCAAGCGGGGCTCTGGCAAGGACTCACACCACCCGGCAAGAACTGCTCACTAC
GGCTCCCTGCCCCAGAAGTCACACGGCCGGACCCAAGATGAAAACCCCGTAGTCCACTTCTTCAAGAACA
TTGTGACGCCTCGCACACCACCCCCGTCGCAGGGAAAGGGGAGAGGACTGTCCCTGAGCAGATTTAGCTG
GGGGGCCGAAGGCCAGAGACCAGGATTTGGCTACGGAGGCAGAGCGTCCGACTATAAATCGGCTCACAAG
GGATTCAAGGGAGTCGATGCCCAGGGCACGCTTTCCAAAATTTTTAAGCTGGGAGGAAGAGATAGTCGCT
CTGGATCACCCATGGCTAGACGCTGA  (SEQ ID NO: 22)
```

[0060] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 3, protein

```
  1 masqkrpsqr hgskylatas tmdharhgfl prhrdtgild sigrffggdr gapkrgsgkd
 61 shhpartahy gslpqkshgr tqdenpvvhf fknivtprtp ppsqgkgrgl slsrfswgae
121 gqrpgfgygg rasdyksahk gfkgvdaqgt lskifklggr dsrsgspmar r (SEQ ID
NO: 23)
```

[0061] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 4, cDNA

```
ATGGCGTCACAGAAGAGACCCTCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACC
ATGCCAGGCATGGCTTCCTCCCAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGG

CGGTGACAGGGGTGCGCCCAAGCGGGGCTCTGGCAAGGACTCACACCACCCGGCAAGAACTGCTCACTAC
GGCTCCCTGCCCCAGAAGTCACACGGCCGGACCCAAGATGAAAACCCCGTAGTCCACTTCTTCAAGAACA
TTGTGACGCCTCGCACACCACCCCCGTCGCAGGGAAAGGGGGCCGAAGGCCAGAGACCAGGATTTGGCTA
CGGAGGCAGAGCGTCCGACTATAAATCGGCTCACAAGGGATTCAAGGGAGTCGATGCCCAGGGCACGCTT
TCCAAAATTTTTAAGCTGGGAGGAAGAGATAGTCGCTCTGGATCACCCATGGCTAGACGCTGA  (SEQ ID
NO: 24)
```

[0062] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 4, protein

```
  1 masqkrpsqr hgskylatas tmdharhgfl prhrdtgild sigrffggdr gapkrgsgkd
 61 shhpartahy gslpqkshgr tqdenpvvhf fknivtprtp ppsqgkgaeg qrpgfgyggr
121 asdyksahkg fkgvdaqgtl skifklggrd srsgspmarr (SEQ ID NO: 25)
```

[0063] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 8, cDNA

```
ATGGGAAACCACGCAGGCAAACGAGAATTAAATGCCGAGAAGGCCAGTACGAATAGTGAAACTAACAGAG
GAGAATCTGAAAAAAAGAGAAACCTGGGTGAACTTTCACGGACAACCTCAGAGGACAACGAAGTGTTCGG
AGAGGCAGATGCGAACCAGAACAATGGGACCTCCTCTCAGGACACAGCGGTGACTGACTCCAAGCGCACA
GCGGACCCGAAGAATGCCTGGCAGGATGCCCACCCAGCTGACCCAGGGAGCCGCCCCCACTTGATCCGCC
TCTTTTCCCGAGATGCCCCGGGGAGGGAGGACAACACCTTCAAAGACAGGCCCTCTGAGTCCGACGAGCT
CCAGACCATCCAAGAAGACAGTGCAGCCACCTCCGAGAGCCTGGATGTGATGGCGTCACAGAAGAGACCC
TCCCAGAGGCACGGATCCAAGTACCTGGCCACAGCAAGTACCATGGACCATGCCAGGCATGGCTTCCTCC
CAAGGCACAGAGACACGGGCATCCTTGACTCCATCGGGCGCTTCTTTGGCGGTGACAGGGGTGCGCCCAA
GCGGGGCTCTGGCAAGGTGAGCTCTGAGGAGTAG (SEQ ID NO: 26)
```

[0064] Exemplary Homo sapiens myelin basic protein (MBP), transcript variant 8, protein

```
  1 mgnhagkrel naekastnse tnrgesekkr nlgelsrtts ednevfgead angngtssq
```

```
  1 mglmagkrcl lackubthse chrgebekkl hlgeisrtes eaneviqead anqhhgebsq
 61 dtavtdskrt adpknawqda hpadpgsrph lirlfsrdap gredntfkdr psesdelqti
121 qedsaatses ldvmasqkrp sqrhgskyla tastmdharh gflprhrdtg ildsigrffg
181 gdrgapkrgs gkvssee (SEQ ID NO: 27)
```

[0065] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 1, cDNA

```
ATGGGCTTGTTAGAGTGCTGTGCAAGATGTCTGGTAGGGGCCCCCTTTGCTTCCCTGGTGGCCACTGGAT
TGTGTTTCTTTGGGGTGGCACTGTTCTGTGGCTGTGGACATGAAGCCCTCACTGGCACAGAAAAGCTAAT
TGAGACCTATTTCTCCAAAAACTACCAAGACTATGAGTATCTCATCAATGTGATCCATGCCTTCCAGTAT
GTCATCTATGGAACTGCCTCTTTCTTCTTCCTTTATGGGGCCCTCCTGCTGGCTGAGGGCTTCTACACCA
CCGGCGCAGTCAGGCAGATCTTTGGCGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGCAACGGT
AACAGGGGGCCAGAAGGGGAGGGGTTCCAGAGGCCAACATCAAGCTCATTCTTTGGAGCGGGTGTGTCAT
TGTTTGGGAAAATGGCTAGGACATCCCGACAAGTTTGTGGGCATCACCTATGCCCTGACCGTTGTGTGGC
TCCTGGTGTTTGCCTGCTCTGCTGTGCCTGTGTACATTTACTTCAACACCTGGACCACCTGCCAGTCTAT
TGCCTTCCCCAGCAAGACCTCTGCCAGTATAGGCAGTCTCTGTGCTGATGCCAGAATGTATGGTGTTCTC
CCATGGAATGCTTTCCCTGGCAAGGTTTGTGGCTCCAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCC
AAATGACCTTCCACCTGTTTATTGCTGCATTTGTGGGGGCTGCAGCTACACTGGTTTCCCTGCTCACCTT
CATGATTGCTGCCACTTACAACTTTGCCGTCCTTAAACTCATGGGCCGAGGCACCAAGTTCTGA (SEQ ID
NO: 28)
```

[0066] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 1, protein

```
  1 mglleccarc lvgapfaslv atglcffgva lfcgcgheal tgtekliety fsknyqdyey
 61 linvihafqy viygtasfff lygalllaeg fyttgavrqi fgdykticg kglsatvtgg
121 qkgrgsrgqh qahslervch clgkwlghpd kfvgityalt vvwllvfacs avpvyiyfnt
181 wttcqsiafp sktsasigsl cadarmygvl pwnafpgkvc gsnllsickt aefqmtfhlf
241 iaafvgaaat lvsllfmia atynfavlkl mgrgtkf (SEQ ID NO: 29)
```

[0067] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 2, cDNA

```
ATGGGCTTGTTAGAGTGCTGTGCAAGATGTCTGGTAGGGGCCCCCTTTGCTTCCCTGGTGGCCACTGGAT
TGTGTTTCTTTGGGGTGGCACTGTTCTGTGGCTGTGGACATGAAGCCCTCACTGGCACAGAAAAGCTAAT
TGAGACCTATTTCTCCAAAAACTACCAAGACTATGAGTATCTCATCAATGTGATCCATGCCTTCCAGTAT
GTCATCTATGGAACTGCCTCTTTCTTCTTCCTTTATGGGGCCCTCCTGCTGGCTGAGGGCTTCTACACCA
CCGGCGCAGTCAGGCAGATCTTTGGCGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGCAACGTT
TGTGGGCATCACCTATGCCCTGACCGTTGTGTGGCTCCTGGTGTTTGCCTGCTCTGCTGTGCCTGTGTAC
ATTTACTTCAACACCTGGACCACCTGCCAGTCTATTGCCTTCCCCAGCAAGACCTCTGCCAGTATAGGCA
GTCTCTGTGCTGATGCCAGAATGTATGGTGTTCTCCCATGGAATGCTTTCCCTGGCAAGGTTTGTGGCTC
CAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCCAAATGACCTTCCACCTGTTTATTGCTGCATTTGTG
GGGGCTGCAGCTACACTGGTTTCCCTGCTCACCTTCATGATTGCTGCCACTTACAACTTTGCCGTCCTTA
AACTCATGGGCCGAGGCACCAAGTTCTGA  (SEQ ID NO: 30)
```

[0068] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 2, protein

```
  1 mglleccarc lvgapfaslv atglcffgva lfcgcgheal tgtekliety fsknyqdyey
 61 linvihafqy viygtasfff lygalllaeg fyttgavrqi fgdykticg kglsatfvgi
121 tyaltvvwll vfacsavpvy iyfntwttcq siafpsktsa sigslcadar mygvlpwnaf
181 pgkvcgsnll sicktaefqm tfhlfiaafv gaaatlvsll tfmiaatynf avlklmgrgt
241 kf (SEQ ID NO: 31)
```

[0069] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 3, cDNA

```
ATGGGCTTGTTAGAGTGCTGTGCAAGATGTCTGGTAGGGGCCCCCTTTGCTTCCCTGGTGGCCACTGGAT
TGTGTTTCTTTGGGGTGGCACTGTTCTGTGGCTGTGGACATGAAGCCCTCACTGGCACAGAAAAGCTAAT
TGAGACCTATTTCTCCAAAAACTACCAAGACTATGAGTATCTCATCAATGTGATCCATGCCTTCCAGTAT
GTCATCTATGGAACTGCCTCTTTCTTCTTCCTTTATGGGGCCCTCCTGCTGGCTGAGGGCTTCTACACCA
CCGGCGCAGTCAGGCAGATCTTTGGCGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGCAACGGT
AACAGGGGGCCAGAAGGGGAGGGGTTCCAGAGGCCAACATCAAGCTCATTCTTTGGAGCGGGTGTGTCAT
TGTTTGGGAAAATGGCTAGGACATCCCGACAAGTTTGTGGGCATCACCTATGCCCTGACCGTTGTGTGGC
TCCTGGTGTTTGCCTGCTCTGCTGTGCCTGTGTACATTTACTTCAACACCTGGACCACCTGCCAGTCTAT
TGCCTTCCCCAGCAAGACCTCTGCCAGTATAGGCAGTCTCTGTGCTGATGCCAGAATGTATGGTGTTCTC
CCATGGAATGCTTTCCCTGGCAAGGTTTGTGGCTCCAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCC
```

AAATGACCTTCCACCTGTTTATTGCTGCATTTGTGGGGGCTGCAGCTACACTGGTTTCCCTGCTCACCTT
CATGATTGCTGCCACTTACAACTTTGCCGTCCTTAAACTCATGGGCCGAGGCACCAAGTTCTGA (SEQ ID
NO: 32)

[0070] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 3, protein

```
  1 mglleccarc lvgapfaslv atglcffgva lfcgcgheal tgtekliety fsknyqdyey
 61 linvihafqy viygtasfff lygalllaeg fyttgavrqi fgdykticg kglsatvtgg
121 qkgrgsrgqh qahslervch clgkwlghpd kfvgityalt vvwllvfacs avpvyiyfnt
181 wttcqsiafp sktsasigsl cadarmygvl pwnafpgkvc gsnllsickt aefqmtfhlf
241 iaafvgaaat lvsllmfmia atynfavlkl mgrgtkf (SEQ ID NO: 33)
```

[0071] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 4, cDNA

```
ATGGACTATGAGTATCTCATCAATGTGATCCATGCCTTCCAGTATGTCATCTATGGAACTGCCTCTTTCT
TCTTCCTTTATGGGGCCCTCCTGCTGGCTGAGGGCTTCTACACCACCGGCGCAGTCAGGCAGATCTTTGG
CGACTACAAGACCACCATCTGCGGCAAGGGCCTGAGCGCAACGGTAACAGGGGGCCAGAAGGGGAGGGGT
TCCAGAGGCCAACATCAAGCTCATTCTTTGGAGCGGGTGTGTCATTGTTTGGGAAAATGGCTAGGACATC
CCGACAAGTTTGTGGGCATCACCTATGCCCTGACCGTTGTGTGGCTCCTGGTGTTTGCCTGCTCTGCTGT
GCCTGTGTACATTTACTTCAACACCTGGACCACCTGCCAGTCTATTGCCTTCCCCAGCAAGACCTCTGCC
AGTATAGGCAGTCTCTGTGCTGATGCCAGAATGTATGGTGTTCTCCCATGGAATGCTTTCCCTGGCAAGG
TTTGTGGCTCCAACCTTCTGTCCATCTGCAAAACAGCTGAGTTCCAAATGACCTTCCACCTGTTTATTGC
TGCATTTGTGGGGGCTGCAGCTACACTGGTTTCCCTGCTCACCTTCATGATTGCTGCCACTTACAACTTT
GCCGTCCTTAAACTCATGGGCCGAGGCACCAAGTTCTGA (SEQ ID NO: 34)
```

[0072] Exemplary Homo sapiens proteolipid protein 1 (PLP1), transcript variant 4, protein

```
  1 mdyeylinvi hafqyviygt asffflygal llaegfyttg avrqifgdyk tticgkglsa
 61 tvtggqkgrg srgqhqahsl ervchclgkw lghpdkfvgi tyaltvvwll vfacsavpvy
121 iyfntwttcq siafpsktsa sigslcadar mygvlpwnaf pgkvcgsnll sicktaefqm
181 tfhlfiaafv gaaatlvsll tfmiaatynf avlklmgrgt kf (SEQ ID NO: 35)
```

[0073] In some options, the rAAV nucleic acid vector is encapsidated by a rAAV particle as described herein. The rAAV particle may be of any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10), including any derivative (including non-naturally occurring variants of a serotype) or pseudotype. In some options, the rAAV particle is an AAV8 particle, which may be pseudotyped with AAV2 ITRs. Non-limiting examples of derivatives and pseudotypes include AAV2-AAV3 hybrid, AAVrh.10, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV-HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8, AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShH10, AAV2 (Y->F), AAV8 (Y733F), AAV2.15, AAV2.4, AAVM41, and AAVr3.45. Such AAV serotypes and derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699-708. doi: 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan A1, Schaffer DV, Samulski RJ.). In some options, the rAAV particle is a pseudotyped rAAV particle, which comprises (a) a nucleic acid vector comprising ITRs from one serotype (e.g., AAV2) and (b) a capsid comprised of capsid proteins derived from another serotype (e.g., AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10). Methods for producing and using pseudotyped rAAV vectors are known in the art (see, e.g., Duan et al., J. Virol., 75:7662-7671, 2001; Halbert et al., J. Virol., 74:1524-1532, 2000; Zolotukhin et al., Methods, 28:158-167, 2002; and Auricchio et al., Hum. Molec. Genet., 10:3075-3081, 2001).

[0074] Exemplary rAAV nucleic acid vectors useful according to the disclosure include single-stranded (ss) or self-complementary (sc) AAV nucleic acid vectors, such as single-stranded or self-complementary recombinant viral genomes.

[0075] Methods of producing rAAV particles and nucleic acid vectors are also known in the art and commercially available (see, e.g., Zolotukhin et al. Production and purification of serotype 1,2, and 5 recombinant adeno-associated viral vectors. Methods 28 (2002) 158-167; and U.S. Patent Publication Numbers US20070015238 and US20120322861; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, a plasmid containing the nucleic acid vector sequence may be combined with one or more helper plasmids, e.g., that contain a rep gene (e.g., encoding Rep78, Rep68, Rep52 and Rep40) and a cap gene (encoding VP1, VP2, and VP3, including a modified VP3 region as described herein), and transfected into a producer cell line such that the rAAV particle can be packaged and subsequently purified.

[0076] In some options, the one or more helper plasmids includes a first helper plasmid comprising a rep gene and a cap gene and a second helper plasmid comprising a E1a gene, a E1b gene, a E4 gene, a E2a gene, and a VA gene. In some options, the rep gene is a rep gene derived from AAV2 and the cap gene is derived from AAV2 and includes modifications to the gene in order to produce a modified capsid protein described herein. Helper plasmids, and methods of making such plasmids, are known in the art and commercially available (see, e.g., pDM, pDG, pDP1rs, pDP2rs, pDP3rs, pDP4rs, pDP5rs, pDP6rs, pDG(R484E/R585E), and pDP8.ape plasmids from PlasmidFactory, Bielefeld, Germany; other products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; pxx6; Grimm et al. (1998), Novel Tools for Production and Purification of Recombinant Adenoassociated Virus Vectors, Human Gene Therapy, Vol. 9, 2745-2760; Kern, A. et al. (2003), Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids, Journal of Virology, Vol. 77, 11072-11081.; Grimm et al. (2003), Helper Virus-Free, Optically Controllable, and Two-Plasmid-Based Production of Adeno-associated Virus Vectors of Serotypes 1 to 6, Molecular Therapy,Vol. 7, 839-850; Kronenberg et al. (2005), A Conformational Change in the Adeno-Associated Virus Type 2 Capsid Leads to the Exposure of Hidden VP1 N Termini , Journal of Virology, Vol. 79, 5296-5303; and Moullier, P. and Snyder, R.O. (2008), International efforts for recombinant adeno-associated viral vector reference standards, Molecular Therapy, Vol. 16, 1185-1188).

[0077] An exemplary, non-limiting, rAAV particle production method is described next. One or more helper plasmids are produced or obtained, which comprise rep and cap ORFs for the desired AAV serotype and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The cap ORF may also comprise one or more modifications to produce a modified capsid protein as described herein. HEK293 cells (available from ATCC®) are transfected via CaPO4-mediated transfection, lipids or polymeric molecules such as Polyethylenimine (PEI) with the helper plasmid(s) and a plasmid containing a nucleic acid vector described herein. The HEK293 cells are then incubated for at least 60 hours to allow for rAAV particle production. Alternatively, in another example Sf9-based producer stable cell lines are infected with a single recombinant baculovirus containing the

nucleic acid vector. As a further alternative, in another example HEK293 or BHK cell lines are infected with a HSV containing the nucleic acid vector and optionally one or more helper HSVs containing rep and cap ORFs as described herein and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The HEK293, BHK, or Sf9 cells are then incubated for at least 60 hours to allow for rAAV particle production. The rAAV particles can then be purified using any method known the art or described herein, e.g., by iodixanol step gradient, CsCl gradient, chromatography, or polyethylene glycol (PEG) precipitation.

[0078] As used herein, the terms "engineered" and "recombinant" cells are intended to refer to a cell into which an exogenous polynucleotide segment (such as DNA segment that leads to the transcription of a biologically active molecule) has been introduced. Therefore, engineered cells are distinguishable from naturally occurring cells, which do not contain a recombinantly introduced exogenous DNA segment. Engineered cells are, therefore, cells that comprise at least one or more heterologous polynucleotide segments introduced through the hand of man.

[0079] To express a therapeutic agent in accordance with the present disclosure one may prepare a tyrosine capsid-modified rAAV particle containing an expression vector that comprises a therapeutic agent-encoding nucleic acid segment under the control of one or more promoters. To bring a sequence "under the control of' a promoter, one positions the 5' end of the transcription initiation site of the transcriptional reading frame generally between about 1 and about 50 nucleotides "downstream" of (*i.e.,* 3' of) the chosen promoter. The "upstream" promoter stimulates transcription of the DNA and promotes expression of the encoded polypeptide. This is the meaning of "recombinant expression" in this context. Particularly preferred recombinant nucleic acid vector constructs are those that comprise an rAAV nucleic acid vector that contains a therapeutic gene of interest operably linked to one or more promoters that is capable of expressing the gene in one or more selected mammalian cells. Such nucleic acid vectors are described in detail herein.

[0080] The genetic constructs of the present disclosure may be prepared in a variety of compositions, and may also be formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects. The rAAV molecules of the present disclosure and compositions comprising them provide new and useful therapeutics for the treatment, control, and amelioration of symptoms of a variety of disorders, diseases, injury, and/or dysfunctions of the mammalian nervous system, and in particular, in the treatment or amelioration of MS.

[0081] In some options, the number of rAAV particles administered to a subject may be on the order ranging from 10^6 to 10^{14} particles/ml or 10^3 to 101^5 particles/ml, or any values therebetween for either range, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} particles/ml. In one option, rAAV particles of higher than 10^{13} particles/ml may be administered. In some options, the number of rAAV particles administered to a subject may be on the order ranging from 10^6 to 10^{14} vector genomes(vgs)/ml or 10^3 to 10^{15} vgs/ml, or any values therebetween, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or

10^{14} vgs/ml. In one option, rAAV particles of higher than 10^{13} vgs/ml are administered. The rAAV particles can be administered as a single dose, or divided into two or more administrations as may be required to achieve therapy of the particular disease or disorder being treated. In some options, 0.0001 ml to 10 mls, e.g., 0.001ml, 0.01ml, 0.1ml, 1 ml, 2ml, 5ml or 10 ml, are delivered to a subject. In some options, the number of rAAV particles administered to a subject may be on the order ranging from 10^{6}-10^{14} vg/kg, or any values therebetween, such as for example, about 10^{6}, 10^{7}, 10^{8}, 10^{9}, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} vgs/mg.

[0082] In some options, the disclosure provides formulations of one or more viral-based compositions disclosed herein in pharmaceutically acceptable solutions for administration to a cell or an animal, either alone or in combination with one or more other modalities of therapy, and in particular, for therapy of human cells, tissues, and diseases affecting man.

[0083] If desired, rAAV particles described herein may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically-active agents, including one or more systemic or topical administrations of therapeutic polypeptides, biologically active fragments, or variants thereof. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The rAAV particles may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein.

[0084] Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravitreal, intraocular, intravenous, intranasal, intra-articular, and intramuscular administration and formulation.

[0085] Typically, these formulations may contain at least about 0.1% of the therapeutic agent (e.g., rAAV particle) or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of therapeutic agent(s) in each therapeutically-useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0086] In certain circumstances it will be desirable to deliver rAAV particles in suitably formulated pharmaceutical compositions disclosed herein either subcutaneously, intraocularly,

intravitreally, parenterally, subcutaneously, intravenously, intracerebro-ventricularly, intramuscularly, intrathecally, orally, intraperitoneally, by oral or nasal inhalation, or by direct injection to one or more cells, tissues, or organs by direct injection. The pharmaceutical forms of the compositions suitable for injectable use include sterile aqueous solutions or dispersions. In some options, the form is sterile and fluid to the extent that easy syringability exists. In some options, the form is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, saline, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[0087] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the rAAV particle is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers. Other exemplary carriers include phosphate buffered saline, HEPES-buffered saline, and water for injection, any of which may be optionally combined with one or more of calcium chloride dihydrate, disodium phosphate anhydrous, magnesium chloride hexahydrate, potassium chloride, potassium dihydrogen phosphate, sodium chloride, or sucrose.

[0088] The compositions of the present disclosure can be administered to the subject being treated by standard routes including, but not limited to, pulmonary, intranasal, oral, inhalation, parenteral such as intravenous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intravitreal, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection. In some options, the composition is administered intravenously, by hepatic artery infusion, portal vein injection, or intrasplenic injection. In some options, the composition comprises a AAV8 rAAV particle comprising a rAAV nucleic acid vector as described herein, and the composition is administered intravenously.

[0089] For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intravitreal, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage may occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration,

preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by, e.g., FDA Office of Biologics standards.

[0090] Sterile injectable solutions may be prepared by incorporating the rAAV particles in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, exemplary methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0091] The amount of rAAV particle compositions and time of administration of such compositions will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the disclosed compositions may be achieved by a single administration, such as for example, a single injection of sufficient numbers of viral particles to provide therapeutic benefit to the patient undergoing such treatment. Alternatively, in some circumstances, it may be desirable to provide multiple, or successive administrations of the compositions, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

[0092] The composition may include rAAV particles or nucleic acid vectors either alone, or in combination with one or more additional active ingredients, which may be obtained from natural or recombinant sources or chemically synthesized.

[0093] In accordance with the present disclosure, polynucleotides, nucleic acid segments, nucleic acid sequences, and the like, include, but are not limited to, DNAs (including and not limited to genomic or extragenomic DNAs), genes, peptide nucleic acids (PNAs), RNAs (including, but not limited to, rRNAs, mRNAs and tRNAs), nucleosides, and suitable nucleic acid segments either obtained from natural sources, chemically synthesized, modified, or otherwise prepared or synthesized in whole or in part by the hand of man.

[0094] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and compositions similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and compositions are described herein. For purposes of the present disclosure, the following terms are defined below:

[0095] The term "subject," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the present disclosure can be provided. Mammalian species that can benefit from the disclosed methods of treatment

include, but are not limited to, humans; apes; chimpanzees; orangutans; monkeys; domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters. In some options, the subject has, is suspected of having, is at risk for developing, or has been diagnosed with an autoimmune disease or disorder, such as multiple sclerosis, disseminated sclerosis, or encephalomyelitis disseminata. In some options, the subject has an autoimmune disease or disorder, such as multiple sclerosis, disseminated sclerosis, or encephalomyelitis disseminata. Other exemplary autoimmune diseases include type 1 diabetes, Grave's disease, arthritis (e.g., rheumatoid arthritis or PGIA), autoimmune uveitis, Peripheral Neuropathy, Myasthenia gravis, Lupus, and Crohn's disease. In some options, an autoimmune disease or disorder is associated with an infection (e.g., a microbial or viral infection).

[0096] The term "treatment" or any grammatical variation thereof (e.g., treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, alleviating a symptom of a disease or condition; and/or reducing, suppressing, inhibiting, lessening, ameliorating or affecting the progression, severity, and/or scope of a disease or condition.

[0097] The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect.

[0098] The term "promoter," as used herein refers to a region or regions of a nucleic acid sequence that regulates transcription.

[0099] The term "regulatory element," as used herein, refers to a region or regions of a nucleic acid sequence that regulates transcription. Exemplary regulatory elements include, but are not limited to, enhancers, post-transcriptional elements, transcriptional control sequences, and such like.

[0100] The tern "vector," as used herein, refers to a nucleic acid molecule (typically comprised of DNA) capable of replication in a host cell and/or to which another nucleic acid segment can be operatively linked so as to bring about replication of the attached segment. A plasmid, cosmid, or a virus is an exemplary vector.

[0101] The term "substantially corresponds to," "substantially homologous," or "substantial identity," as used herein, denote a characteristic of a nucleic acid or an amino acid sequence, wherein a selected nucleic acid or amino acid sequence has at least about 70 or about 75 percent sequence identity as compared to a selected reference nucleic acid or amino acid sequence. More typically, the selected sequence and the reference sequence will have at least about 76, 77, 78, 79, 80, 81, 82, 83, 84 or even 85 percent sequence identity, and more preferably, at least about 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95 percent sequence identity. More preferably still, highly homologous sequences often share greater than at least about 96, 97, 98, or 99 percent sequence identity between the selected sequence and the reference sequence to which it was compared.

[0102] The percentage of sequence identity may be calculated over the entire length of the sequences to be compared, or may be calculated by excluding small deletions or additions which total less than about 25 percent or so of the chosen reference sequence. The reference sequence may be a subset of a larger sequence, such as a portion of a gene or flanking sequence, or a repetitive portion of a chromosome. However, in the case of sequence homology of two or more polynucleotide sequences, the reference sequence will typically comprise at least about 18-25 nucleotides, more typically at least about 26 to 35 nucleotides, and even more typically at least about 40, 50, 60, 70, 80, 90, or even 100 or so nucleotides.

[0103] When highly-homologous fragments are desired, the extent of percent identity between the two sequences will be at least about 80%, preferably at least about 85%, and more preferably about 90% or 95% or higher, as readily determined by one or more of the sequence comparison algorithms well-known to those of skill in the art, such as *e.g.,* the FASTA program analysis described by Pearson and Lipman (1988).

[0104] The term "operably linked," as used herein, refers to that the nucleic acid sequences being linked are typically contiguous, or substantially contiguous, and, where necessary to join two protein coding regions, contiguous and in reading frame. However, since enhancers generally function when separated from the promoter by several kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not contiguous.

[0105] The term "biologically active" as used herein, refers to a variant nucleic acid or protein sequence that has substantially the same activity, such as reduction of clinical severity of EAE in a mouse model or induction of T regulatory cells as described in Example 1, Example 2, Example 3, or Example 4 below, as a nucleic acid or protein as described herein (e.g., has substantially the same or the same activity as a MOG, PLP, or MBP nucleic acid or protein described herein).

EXAMPLES

EXAMPLE 1 - RE-ESTABLISHING IMMUNE TOLERANCE TO NEUROANTIGENS BY AAV GENE THERAPY

[0106] The inventors have demonstrated that hepatocyte-restricted expression of an AAV-delivered neuroantigen establishes persistent immunological tolerance mediated by antigen-specific Tregs capable of preventing and reversing EAE in mice. This example describes the development of a protocol that persistently induces Tregs *in vivo* and prevents disease development in a murine model of MS. The example also determines if tolerance can induce remission of pre-existing EAE disease and substantially reduce clinical and tissue-associated pathology.

[0107] Neurodegenerative disease such as Multiple sclerosis (MS) is characterized by chronic infiltration of the CNS by pathogenic autoreactive lymphocytes that recognize neuroantigens. Functional defects in the endogenous regulatory T cells (Tregs) leading to a failure of central and/or peripheral mechanisms required for maintaining immunological tolerance combined with T cells recognizing myelin protein peptides are implicated in the pathogenesis of the disease. In C57BL/6 mice, experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) produces a CD4 T cell-mediated inflammatory CNS disease that serves as a relevant model for MS (FIG. 1 and FIGs. 2A and 2B).

[0108] Hepatic gene transfer with AAV vectors containing liver specific promoters can produce stable transgene expression and induce a robust antigen-specific immune tolerance to a variety of therapeutic proteins. It has been reported that induced Tregs not only suppress cellular immune responses against the transgene product but can also suppress humoral responses. Importantly, it has been shown that immune tolerance established by antigen expression in the liver is maintained even when the antigen was subsequently expressed in a highly immunogenic manner in other organs, such as skeletal muscle or intravenously.

[0109] The development of protocols that stimulate an increase in Treg numbers and/or their function has become a focus in treating autoimmune disease. Many of the beneficial effects of currently approved immune-modulators used in the treatment of MS are associated with restoring Treg homeostasis. This example demonstrates that liver directed AAV gene therapy represents a novel approach to halt disease progression by restoring normal Treg function at disease onset.

[0110] First, an AAV8-MOG vector was generated, and hepatic expression of the transgene in mice was validated by western blot and qPCR analysis (FIG. 4). Next, to determine if hepatic expression of MOG can provide protection against the development of EAE mice were injected with either AAV8-MOG or -GFP vector. 2 weeks later EAE was induced and the mice were monitored and scored according to the classic scale for clinical signs of EAE. Plasma was obtained at 0, 7 and 14 days post EAE or at 0, 11, 19,26, and 35 days post EAE. The results revealed that mice receiving AAV8-MOG were clearly protected from developing EAE. Furthermore, these mice also did not produce any anti-MOG IgG1 or IgG2c autoantibodies. In contrast, those mice receiving the control vector developed severe EAE with elevated antibody titers (FIGs. 7 and 8).

EXAMPLE 2 -THERAPEUTIC MOLECULES FOR AAV-BASED GENE THERAPY OF MS

[0111] This example shows that liver directed gene transfer using an AAV vector expressing a neuro-antigen is capable of suppressing inflammation in the CNS and preventing EAE. Importantly, using AAV to express a full-length neuro-protein will enable greater applicability across MS-associated HLA haplotypes. Ongoing plans are to evaluate reversal of pre-existing EAE and functional analysis of the interplay of effector (Th1/Th17) cells and Tregs.

[0112] Using sequences for full length proteins, avoiding HLA/MHC restrictions

MBP sequence in vector:

MGNHSGKRELSAEKASKDGEIHRGEAGKKRSVGKLSQTASEDSDVFGEADAIQNNGTSAEDT
AVTDSKHTADPKNNWQGAHPADPGNRPHLIRLFSRDAPGREDNTFKDRPSESDELQTIQEDP
TAASGGLDVMASQKRPSQRSKYLATASTMDHARHGFLPRHRDTGILDSIGRFFSGDRGAPKR
GSGKVSSEP* (SEQ ID NO:1)

PLP sequence in vector:

MGLLECCARCLVGAPFASLVATGLCFFGVALFCGCGHEALTGTEKLIETYFSKNYQDYEYLI
NVIHAFQYVIYGTASFFFLYGALLLAEGFYTTGAVRQIFGDYKTTICGKGLSATVTGGQKGR
GSRGQHQAHSLERVCHCLGKWLGHPDKFVGITYALTVVWLLVFACSAVPVYIYFNTWTTCQS
IAFPSKTSASIGSLCADARMYGVLPWNAFPGKVCGSNLLSICKTAEFQMTFHLFIAAFVGAA
(SEQ ID NO:2)

MOG sequence in vector:

MACLWSFSLPSCFLSLLLLLLLQLSCSYAGQFRVIGPGYPIRALVGDEAELPCRISPGKN
ATGMEVGWYRSPFSRVVHLYRNGKDQDAEQAPEYRGRTELLKETISEGKVTLRIQNVRFS
DEGGYTCFFRDHSYQEEAAMELKVEDPFYWVNPGVLTLIALVPTILLQVSVGLVFLFLQH
RLRGKLRAEVENLHRTFDPHFLRVPCWKITLFVIVPVLGPLVALIICYNWLHRRLAGQFL
EELRNPL (SEQ ID NO:3)

EAE inducing peptide in SJL mice PLP $_{139-151}$ HCLGKWLGHPDKF (SEQ ID NO:4)

EAE inducing peptide in C57BL mice NTWTCQSIAFP (SEQ ID NO:5) or PLP$_{178-191}$ NTWTTCQSIAFPSK (SEQ ID NO: 37)

C57BL: MOG35-55 MEVGWYRSPFSRVVHLYRNGK (SEQ ID NO:6)

SJL: MOG92-106 DEGGYTCFFRDHSYQ (SEQ ID NO:7)

EXAMPLE 3 -RAAV8 VECTORS FOR GENE THERAPY OF MS

[0113] AAV8 vectors can stably express a neuro-protein in hepatocytes. AAV8-MOG can prevent the development of EAE, and AAV8-MOG can abrogate clinical symptoms of established EAE.

[0114] This example describes the development of a (pre)clinically relevant therapy using viral gene transfer that will result in the induction and expansion of antigen-specific T cells, re-establishing immunological tolerance as a treatment for multiple sclerosis. The approach has broad application as it uses full length myelin oligodendrocyte glycoprotein (MOG) protein and thus abrogates the needs for identifying HLA/MHC specific epitopes for inducing antigen specific Tregs. The knowledge gained from the work presented here will have the potential of creating a new line of treatment protocols for patients with MS as well as advance the research for both the MS and gene therapy fields.

[0115] Collectively, there is clear rationale for therapeutic approaches that are multifactorial. The present disclosure provides a novel therapy that not only focuses on reducing $CD4^+$ T cells, but that can also target the effect of $CD8^+$ T cells, B cells, and B cell derived components of the immune system. Previous data, suggests that the presentation of neuro-antigens within liver delivered by AAV should have significant clinical relevance as a therapeutic intervention for MS and other autoimmune diseases. This application of gene therapy represents a new and innovative treatment option for MS.

EXPERIMENTAL METHODS

[0116] ***Prevention of EAE:*** C57B1/6 mice will be injected with AAV8-MOG or control vector for hepatocyte-specific expression. At 4 weeks after gene transfer, induction of EAE will begin. Mice will be monitored daily and neurological impairment will be recorded on a detailed clinical scale. Weekly, blood/serum will be collected and analyzed for frequency of activated CD4, CD8, and Tregs by flow cytometric analysis, and α-MOG antibody formation via ELISA. Upon termination, histology of harvested tissues will be evaluated for transgene expression (liver), magnitude and phenotype of infiltration of inflammatory cells, demyelination, and white matter damage (CNS). Establishing a baseline correlation of histology to clinical score (especially in control mice) will be essential for identifying success in subsequent aims.

[0117] ***Reversal of EAE:*** In this study, induction of EAE in mice is first performed. Upon the first signs of EAE, mice will be randomly selected to receive hepatic gene transfer using AAV8-MOG or control vector. A detailed clinical assessment will be recorded daily. At various time points, blood/serum will be collected and analyzed as above. Upon sacrificing, liver and CNS tissue will be harvested and preserved for pathological and histochemical analysis.

[0118] ***Ex vivo functional analysis of tolerogenic Tregs:*** To determine if the AAV8-MOG induced Tg-specific Tregs are immunosuppressive, GFP^+Tregs isolated by FACS from transgenic mice ("$Foxp3^{EGFP}$" B6.Cg-*Foxp3*^{tm2Tch}/J) that received vector 4 wks earlier will be co-cultured with allogeneic splenocytes obtained from 2D2-TCR mice (MOG specific TCR) in the presence of MOG peptide. Cells and culture supernatant will be analyzed for activation, apoptosis, Th1/Th2/Th17 cytokines, or CTL activity via specific assays.

[0119] ***In vivo adoptive transfer of Tregs:*** To test whether the immunosuppressive function of Tg-specific Tregs are able to attenuate disease progression, GFP^+Tregs isolated as above, will be adoptively transferred into (a). naive mice that will subjected to EAE induction 24hrs later AND (b). mice that have undergone MOG induced EAE. These conditions will mirror the events from Aim 1.1 prevention and 1.2 reversal. At various time points, blood/serum will be collected; and liver and CNS tissue will be harvested and analyzed as above.

[0120] The overall theme of the present disclosure is the development of a gene therapy

based method for *in vivo* induction of endogenous antigen (Ag)-specific regulatory T-cells (Tregs) using liver-directed Adeno-associated virus (AAV) gene therapy, as a novel treatment strategy for autoimmune diseases, e.g., multiple sclerosis (MS).

[0121] As noted above, MS is an autoimmune neurodegenerative disease of the central nervous system (CNS) in which the etiology is not well understood. Although, auto-aggressive $CD4^+$ T cells play a central role, the breakdown of immune tolerance mechanisms that permits activation of naive myelin-specific T cells is considered an initial step in the pathogenesis of MS. A number of pivotal studies in rodent models have substantiated that Ag-specific Tregs have a significant role in modulating autoimmune CNS disease and can be highly effective at treating MS.[1-5] Consequentially, there has been a major focus in developing protocols that stimulate Treg numbers and their function. Unfortunately, successful therapeutic use of Tregs has been limited by the lack of safe and effective Ag-specific protocols for isolation and expansion that are suitable for translation.

[0122] Using the AAV gene transfer platform, it has been clearly demonstrated that hepatocyte-restricted transgene expression from an optimized AAV vector can reliably induce immune tolerance to various therapeutic proteins, including coagulation factor IX (F.IX), α-1-antitrypsin, erythropoietin, and lysosomal storage enzymes, among others.[6] Tolerance induction after hepatic gene transfer involves a combination of mechanisms. Importantly, AAV induced tolerance is mediated by Ag-specific $CD4^+CD25^+FoxP3^+$ Tregs, which is critically dependent on achieving and maintaining adequate hepatocyte-restricted transgene expression.[7-9] It has also been demonstrated that AAV induced Tregs can actively suppress antibody formation and cytotoxic $CD8^+$ T cell responses against the transgene product.[7, 10, 11] Tolerized animals fail to form antibodies to the transgene even after subsequent attempts to immunize with protein formulated in adjuvant.[10-12] Efficient hepatic gene transfer induces a TGF-β dependent $CD4^+CD25^+FoxP3^+$ Treg response that confers a dominant state of Ag-specific immune tolerance that is maintained even when the antigen was later introduced in other tissues in a highly immunogenic manner.[7, 12] Induction of programmed cell death of effector T cells further tilts the balance toward tolerance, which is effectively enforced by induced Treg.[13, 14] Published data have demonstrated that hepatic AAV tolerance can also reverse pre-existing immune responses to F.IX in a hemophilia B mouse model.[15] These now well-established concepts have been further supported by results from other laboratories and led to the development of several immune tolerance protocols for genetic diseases. [16-37]

[0123] Over 400,000 people in the United States currently are living with MS, and 10,000 new cases are diagnosed each year. With a 1:600-800 lifetime risk of developing the disease, MS is the most common cause of neurologic disability in young adults between 18 and 45 years of age. This demographic represents the majority of the adult workforce in the United States; therefore, the direct and indirect costs of health care for this population currently are estimated at $12 billion annually.[38]

[0124] ***Multiple sclerosis (MS) is a protracted, immune-mediated disease of the CNS.*** MS is a neuroinflammatory autoimmune disease in which T cell driven inflammation leads to demyelination and damage of axons. Although the exact pathogenesis of MS remains unknown, it is believed that myelin-specific $CD4^+$ T cells play a central role in initiating and orchestrating CNS inflammation. A failure of central and peripheral mechanisms (particularly Tregs) to maintain self-tolerance and control potentially pathogenic auto-reactive lymphocytes is thought to be a key event in the development and pathogenesis ofMS.[4, 39-41] Several studies using *in vitro* suppression assays have documented functional impairments of Tregs from MS patients.[42, 43] Experiments in mice using adoptive transfer of myelin-specific Tregs or Treg depletion have also provided evidence that Tregs can control the development and severity of experimental autoimmune encephalomyelitis (EAE) and accumulate within the CNS during the recovery.[44] It has also been shown that transgenic mice expressing myelin basic protein (MBP) could prevent the onset of EAE disease in mice in a Treg dependent process.[45,46] In fact, the mechanism-of-action for several of the currently approved immune-modulators used in the treatment of MS are associated with restoring Treg homeostasis.[39, 47, 48]

[0125] Cumulatively, the literature clearly supports the concept that Treg cells influence the susceptibility and progression of disease. Recent advances have led to the recognition that Ag-specific Tregs represents an ideal form of cell therapy for MS. However, Tregs are still among the least understood T cell subsets, and consequently the most difficult to use for therapeutic applications.

[0126] ***Gene therapy with AAV vectors induces antigen-specific immune tolerance.*** Gene therapy continues to be a proven and powerful new tool for the treatment of a broad spectrum of diseases.[49] AAV vectors specifically have had great successes with *in vivo* gene transfer to a variety of target tissues.[12] For example, AAV gene transfer to retinal epithelial cells restores vision in children with Leber's Congenital Amaurosis (LCA) and with Choroideremia.[50, 51] An AAV vector for treatment of lipoprotein lipase is the first gene therapy drug approved in the Western world ("Glybera").[52] Gene therapy by hepatic AAV administration has resulted in sustained expression of factor IX (F.IX) at levels of >5% of normal in hemophilia B patients, changing their bleeding phenotype from severe to mild.[48] Hepatic AAV gene transfer promotes tolerance via induction of transgene product-specific Treg, a phenomenon that can be exploited for the treatment of MS.[6, 21, 34, 35, 49, 53]

[0127] Effective therapy for established EAE needs to consider induction of multiple direct and indirect (cross-tolerance) regulatory mechanisms, including the induction of antigen (Ag)-specific $CD4^+CD25^+FoxP3^+$ Tregs across multiple endogenous myelin epitopes (epitope spreading). The literature supports the idea that Tregs are potent suppressors of EAE, and essential to establish disease remission. However, very few studies have addressed how to generate such Ag-specific Tregs that is both reliable and translatable. Based on previous work,

hepatic gene transfer using AAV8 vectors to express full-length myelin-associated proteins will induce Ag-specific Tregs across multiple endogenous epitopes in a manner that has been shown to be safe, feasible, and long lasting. This study is innovative in several respects. (i) This is the first time a clinically proven AAV vector technique is used to re-establish immunological tolerance in the context of an autoimmune disease. (ii) exemplary AAV8 vectors have been designed to express a full-length neuro-protein (myelin oligodendrocyte glycoprotein (MOG) or proteolipid proteins (PLP)) thus abrogating the need for identifying HLA/MHC specific epitopes and enhancing the potential for success. (iii) Based on published data, incorporating transient immune modulation using the FDA approved mTOR inhibitor, rapamycin, should provide a synergistic effect, facilitating tolerance induction to neuroantigens by further tipping the balance from Teff to Treg *in vivo.*[54-56]

[0128] The inventors contemplate that AAV8 liver gene transfer of a neural protein (PLP and MOG) induces activation of Ag-specific Tregs, sufficient to re-establish immune tolerance and abrogate disease progression in the CNS of a murine model for MS.

[0129] The basis for this proposal is founded on precedent, and recently-published studies in which relevant findings are presented in the following:

[0130] Hepatic gene transfer with AAV vectors can reliably induce a robust antigen-specific immune tolerance in experimental animals to a variety of therapeutic proteins.[7, 9, 13, 54, 57] Here, tolerance was characterized by lack of antibody formation, helper T cell response, or CTL response to the transgene product, even after subsequent challenge with protein in adjuvant. Using mice transgenic for a T cell receptor, evidence of anergy and deletion of transgene product-specific CD4 T cells was found.

[0131] That immune tolerance established by hepatic transgene expression is maintained even when the antigen was subsequently expressed in a highly immunogenic manner in other organs, such as skeletal muscle, or even delivered intravenously.[7] These results revealed that liver directed gene therapy could abrogate potential cytotoxic CD8 T cell responses, indicating that the range of immune tolerance extends beyond the level of antigen expression initially achieved by hepatic gene transfer.

[0132] Hepatic AAV gene transfer efficiently and rapidly reversed pre-existing high antibodies titers and provided long-term correction of haemostasis in a murine hemophilia B model. [15, 56] High levels of transgene protein suppressed memory B cells and increased Treg induction, indicating direct and indirect mechanisms of suppression of inhibitor formation. This finding is quite significant since there is an increasing body of evidence that B cells and autoantibodies may play a pathogenic role in demyelinating disease.[58, 59]

[0133] ***Immune tolerance induction by hepatic AAV gene transfer does not require protein to be secreted.*** Although hepatic expression is crucial for tolerance induction, secretion from hepatocytes for systemic delivery of the transgene product is not required.

Expression of a cytoplasmic a neo-antigen in as few as 3% of the hepatocytes is sufficient to induce Tregs and provide long-term suppression of inflammatory responses.[57]

RESULTS

[0134] ***Successfully establishing multiple models of EAE induction:*** EAE is a widely accepted experimental mouse model of multiple sclerosis that is induced in susceptible animals by immunization with central nervous system antigens. EAE is an autoimmune disease that is mediated by $CD4^+$ T helper 1 (T_H1) cells and interleukin-17 producing T_H17 cells that are reactive to components of the myelin sheath. The cells infiltrate the nervous parenchyma, release pro-inflammatory cytokines and chemokines, promote leukocyte infiltration and contribute to demyelination.

[0135] EAE can be induced in various strains of mice using different neuro-proteins emulsified in complete Freud's adjuvant (CFA). Disease progression and pathology manifests differently with each combination. For example, EAE induced by (i) MOG produces encephalitogenic T-cells and demyelinating autoantibodies in C57BL/6 mice. The resulting disease is a chronic-progressive disease characterized by axonal demyelination and white matter lesions in the spinal cord and is generally considered to be a relevant model for human immune-mediated demyelinating disease.[60] EAE can also be induced in SJL (H-2s) mice using the major encephalitogenic *(ii)* PLP peptide ($PLP_{139-151}$). Here the disease is characterized by a relapsing-remitting course of paralysis, which allows assessment of the efficacy of various immune regulatory strategies in a re-occurring disease setting.

[0136] In these studies, the inventors demonstrated the timeline and clinical scoring for successful induction of EAE disease in two different mouse strains. In one experiment, 8-week-old female mice were injected subcutaneously with 200µg myelin peptide emulsified in CFA containing 4mg/ml Mycobacterium tuberculosis. Clinical signs of EAE began 12 days later at which time mice were evaluated twice daily. Mice were scored according to the severity of the clinical signs (FIG. 5A). In a similar experiment, EAE was induced in C57BL/6 mice (n = 5) using MOG in order to develop a chronic progressive EAE disease (FIG. 5B)

[0137] ***Novel AAV8 vectors transduce mouse hepatocytes efficiently and express the delivered neural protein:*** AAV is a non-pathogenic single stranded DNA parvovirus with a genome size of approximately 4.7kb. Serotypes with distinct tissue tropisms have been isolated from multiple vertebrate species, including humans. Viral vectors derived from AAV are devoid of viral genes and instead contain an expression cassette for the gene of interest, which is limited to ~5kb in length. In these studies, an AAV8 serotype vector was chosen because it has strong natural tropism for hepatocytes after peripheral vein administration, avoiding the need for an invasive procedure. Additionally, it fails to transduce professional antigen presenting cells (APCs). The engineered vector constructs include a strong and highly hepatocyte-specific

promoter.[10]

[0138] The newly synthesized vectors were evaluated for transduction efficiency. To demonstrate efficacy, the inventors assessed whether mouse hepatocytes could be transduced and express the neuro-protein transgene following tail vein injection. A group of mice was injected with 1×10^{11} vector particles of AAV8-ApoE/hAAT-MOG. Two weeks later, using liver lysates evidence of hepatic expression of MOG was probed by both western blot and qPCR analysis. The results clearly demonstrate the ability this novel vector to stably produce hepatic expression of the neuro-antigen after liver gene transfer (FIG. 4A and FIG. 4B).

[0139] ***AA V8-MOG produces hepatic transgene expression that can prevent the establishment of EAE:*** Previously others have shown that ectopic expression of a myelin-associated protein using various transient methodologies promoted resistance to EAE.[18, 28, 45, 61] Unfortunately, these prior approaches have not developed into practical therapies for human autoimmune disease. Prior to this disclosure, the ability of AAV liver gene transfer to induce antigen specific suppression of autoimmune disease went untested in the scientific community.

[0140] To further support this disclosure, a pilot study was performed. A small number of mice (n=5) were intravenously injected with 10^{11} vector particles *via* the tail vein with either AAV8-MOG or AAV8-GFP (control) vector. Two weeks later, EAE was induced using MOG in CFA as previously performed. Plasma samples were obtained at 0, 7 and 14 days post EAE induction or at 0, 11, 19,26, and 35 days post EAE induction. The mice that received AAV8-MOG were essentially protected from developing EAE (FIG. 6A, FIG. 6B, and FIG. 6C). In contrast, those mice receiving the control vector developed severe EAE with elevated antibody titers. This data indicates that the vectors described herein not only express in the liver, but also had an immune modulatory effect.

[0141] Active suppression by Tregs plays a key role in the control of auto-reactive T cells and the induction of peripheral tolerance *in vivo.* In particular, the significance of Ag-specific Tregs in conferring resistance to organ-specific autoimmunity and in limiting autoimmune tissue damage has been documented in many disease models, including MS.[44] However, a safe and clinically feasible method for sustained expansion of endogenous Tregs has yet been identified.[41, 44, 60, 63] a treatment protocol based on liver-directed AAV gene therapy can durably induce Ag-specific tolerance, thus having the potential of blocking the pathogenic autoimmune response present in MS and inhibiting disease activity; while avoiding the severe side effects associated with many of the currently used immunotherapies. Based on these and related studies, AAV8-liver gene transfer can restore immunological tolerance against myelin-sheath antigens, such as MOG and PLP, by inducing Ag-specific Tregs *in vivo.*

[0142] ***Experimental approach and methods of analyses:*** This set of experiments tests vector constructs in order to verify efficiency of liver transduction and hepatic expression without adverse effects. Groups of (i) C57BL/6 or (ii) SJL/J mice (7-8 weeks old) will be injected

with the 10^{11} vector particles (vp) (effective dose of vector as previously determined) of (i) AAV8-MOG or (ii) AAV8-PLP (respectively), or control (irrelevant transgene, GFP) intravenously *via* the tail vein. Beginning on day 0, blood will be collected every 2 weeks and analyzed for the frequency of various T cell populations using standard markers of T cells phenotype (including, but not limited to, CD4, CD8, FoxP3, CD25, CD62L, CD44). Humoral immune responses (e.g. α-IgG1, -IgG2a, -IgG2c responses) may be determined *via* antigen specific ELISA. At 14 days post gene transfer, half of the mice from each group may be randomly selected and humanely euthanized. Tissues (blood, liver, spleen, and CNS (brain/spinal cord)) may be harvested for analysis. Hepatic transgene expression levels may be determined at the mRNA level using real-time quantitative PCR. Absolute and relative hepatic protein levels of the transgene will also be determined via western blot using liver lysates. At 90 days post injection, the remainder of the mice may be processed similarly to establish sustained transgene expression. Additionally, some mice may be subjected to EAE induction at various time points after vector administration and evaluated for prevention of disease, as described in preliminary data. Aliquots of the collected tissue samples may be archived as a reference material.

[0143] ***In vitro functional suppression analysis of Ag-specific Tregs induced by AAV8 hepatic gene transfer.*** Splenic Tregs ($CD4^+CD25^+$) may be magnetically sorted from mice that received (i) AAV8-MOG or (ii) AAV8-PLP or AAV8-GFP (control) vector and co-cultured with graded numbers of CFSE labeled cells obtained from 2D2-TCR mice (this C57B1/6 mouse line expresses a TCR which recognize MOG_{35-55} in the context of H-2 IA^b) or splenocytes harvested and labeled from SJL mice that have been previously immunized with PLP/adjuvant in the presence of anti-CD3/CD28 coated beads (provides APC independent/non-specific activation of Teff). Treg mediated suppression of proliferating effector cells may be determined by flow cytometry. Cell-culture supernatants may be analyzed for Th1/Th2/Th17 cytokines via specific assays. Results may be compared with data from naive and EAE induced mice (in which many $CD4^+CD25^+$ cells should represent activated effector rather than Treg). In combination, these studies will demonstrate Ag-specific functional suppression from the vector induced Tregs compared to controls.

[0144] Based on the initial data and published studies, the inventors expect maximal transgene expression by 2 weeks, that remains fairly unchanged over time, thus indicating stable transduction of hepatocytes.[10] Since the vector constructs have been purposely designed to express full length MOG or PLP and include a strong hepatocyte promoter, it is also expected that AAV8 vector-mediated expression may be constrained to the hepatocytes and not secreted. Sequestering the transgene will constrain pathological consequences of freely circulating AAV-derived neuroantigen. Lastly, the inventors do not expect inflammatory responses in any tissues and analysis of liver enzymes (ALT/AST) should demonstrate an absence of hepatotoxicity. Furthermore, based on pilot studies, it is expected that vector administration prior to EAE induction will prevent disease development. A positive outcome would also be the absence/significant reduction in antigen specific antibody responses. Lastly, results from the Treg suppression assays are expected to show that suppression induced by

hepatic transgene expression is facilitated by activation of Ag-specific Tregs.

[0145] Even though the literature overwhelmingly supports the idea that Tregs are potent suppressors of EAE and are the driving force to switch from disease progression to remission, very few studies in the past have addressed how to generate such Ag-specific Tregs that is both safe and effective.[64] In theory, this could be achieved by two approaches. The first would be to isolate Tregs, expand their numbers *ex vivo* then reintroduce them, with the idea that an increase in overall frequency of polyclonal Tregs might influence ongoing disease. In 2004, Bluestone's group in a type-1 diabetes model provided initial proof of principle for this approach.[65] More recently, others have further shown that using expanded Tregs from myelin-specific transgenic TCR mice is more effective.[64] The second approach is to administer a suitable treatment that promotes the expansion of Treg numbers and/or function *in vivo.* Recent reports have described the use of various compounds (e.g. nanoparticles/small molecules) to enhance Treg function in EAE, while others try to augment antigen presentation in order to generate Tregs.[64, 66] In the end, a reliable and translatable method for induction of the disease relevant Ag-specific Tregs is still lacking-until now. This proposal presents a methodology that will provide a durable method for the continued *in vivo* induction of endogenous Ag-specific Tregs. Based on previous work, hepatic gene transfer using AAV8 vectors expressing full-length MOG or PLP should induce Ag-specific Tregs across multiple endogenous myelin epitopes in a manner that has been shown to be safe, feasible, and long-lasting.

[0146] ***Experimental approach and methods of analyses.*** Here mice will first under go active induction of EAE using either (i) MOG or (ii) PLP. At the first clinical signs of EAE, in MOG induced chronic-progressive mice, or at the peak of disease, in PLP induced relapsing-remitting mice, AAV8-MOG or AAV8-PLP vector (respectively) or AAV8-GFP for control mice may be given. Mice may be clinically scored by weight and neurological deficit 2x daily. Blood may be collected and analyzed for humoral (IgG) responses as before. At ~45 days, each cohort mice may be perfused and randomly subdivided into 2 groups. Group 1 will have brain, spinal cord, and liver tissues harvested and preserved for histopathological and immunofluorescent analysis. Infiltrating lymphocytes may be isolated from the brain and spinal cords from mice in Group 2 (as previously described[67]). The frequency of various T cell populations may be analyzed using standard markers of T cells (including, but not limited to, CD4, CD8, FoxP3, CD25, CD62L, CD44, CTLA-4, CD103). Liver tissue may be subjected to transcriptional and protein analysis as shown. Results may be compared to control mice and reference material. Portions of the tissue may also be archived for future studies.

[0147] It is expected that therapeutic treatment with a single injection of AAV8-MOG or - PLP vector at the onset or peak of the disease will result in a dramatic remission in clinical impairment. There should be a concurrent reduction in antibody titers and/or frequency of B cell responses to the EAE inducing peptide, as compared to control-vector treated mice. CNS inflammation is characteristic of EAE, and the degree of lymphocyte infiltration correlates with disease progression; whereas, the presence of Tregs in the CNS during EAE has been

associated with diminished inflammation and resolution of clinical disease.[68] Hence, it is expected that a significant reduction of inflammatory infiltrates in the CNS of vector treated mice upon histopathological analysis. This would suggest that the induced Ag-specific Tregs migrating to the site of CNS damage are protective and are capable limiting damage mediated by effector T cells. Additionally, the natural relapsing-remitting nature of PLP induced EAE may be exploited by timing the injection of the AAV8-PLP vector so that the peak effects of induced tolerance correspond to when the disease is relatively quiescent (remitting).

[0148] On the other hand, mice that receive MOG for EAE induction begin showing neurological impairments after ~12 days that progressively escalate. In this scenario, it is possible that some level of inflammation will still be present, although; the phenotypic analysis of the T cells populations show that absolute numbers of T cells infiltrating the CNS to be lower with a greater Treg:Teff ratio.

[0149] ***Transient immunosuppression using rapamycin.*** Rapamycin readily crosses the BBB thus exerting direct effects within the CNS. Blocking the activation of the mTOR pathway, rapamycin prevents activation of T cells by inhibiting their response to IL-2 thus preventing Ag-induced proliferation of Teff, while selectively allowing expansion of functional $CD4^+CD25^+FoxP3^+$ Tregs. In EAE, rapamycin is effective in preventing the onset of disease, however; suppression of established disease is only maintained with continued use.[69] In a further series of experiments, vector treated mice are transiently immunosuppressed. Groups of mice are then injected with AAV8-MOG, -PLP, -GFP or PBS at specific time-points that correspond to either initial onset or peak of disease. Concurrently mice receive intraperitoneal rapamycin (1 mg/kg), or PBS (sham control) daily for 14 consecutive days.[69] At specific time points corresponding to pre- and post-treatment and significant changes in clinical scoring, tissues and lymphocytes may be harvested from the CNS and spleen from randomly selected mice. Histopathological changes within the tissues can then be identified. Isolated cells are then phenotyped and the frequency of Tregs and Teff from the different compartments may be determined and compared to control groups to validate the efficacy of rapamycin co-treatment.

[0150] Regardless of AAV8 administration, treatment with rapamycin alone is expected to transiently produce a rapid reduction in the clinical presentation of EAE because it selectively inhibits Teff proliferation.[69] However, when used in conjunction with AAV8 liver gene transfer, rapamycin treatment has a synergistic effect that results in an increase in vector induced Ag-specific $FoxP3^+$ Tregs (since they are less sensitive to mTOR signaling inhibition) with a corresponding decrease in effector T cells.[70] The shift to tolerance is further potentiated by the fact Tregs have been shown to mediate selective inhibition of antigen-specific Th1 cells in the CNS of EAE.[71]

[0151] When considering the data clearly supports the ability of AAV liver gene transfer to induce Ag-specific Tregs and invoke immune tolerance; and that accumulation of Tregs in the CNS during the recovery phase of EAE has been a consistent finding in actively induced

models, it seems unlikely that the present therapy would not have, at least to some degree, a clinical or pathological benefit.[3, 64, 71, 72]

[0152] In conclusion, the therapeutic regimen presented here addresses an unmet need by providing an effective treatment for diseases such as MS using a gene therapy approach. Using the AAV vector platform disclosed herein to deliver full-length proteins offers a superior HLA independent approach for Ag-specific Treg induction compared to other *ex vivo* or epitope restricted Treg mediated therapies. Additionally, AAV gene transfer results in continuous Treg generation because of the long-term hepatocyte expression of transgene.[73]

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[0153]

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EXAMPLE 4 - FURTHER DATA FROM EAE MOUSE MODEL AND ASSESSMENT OF OTHER PROTEINS

[0154] Animals were injected intravenously via tail vein with 10^{11} vector particles of AAV8-apoE/hAAT-MOG. The MOG sequence used was murine MOG. It was shown that MOG transgene was expressed in the liver as evidenced increased amounts of MOG protein (FIG. 11) in samples from the liver.

[0155] To ensure that AAV did not interfere with the development or progression of EAE in the mouse model described in the other Examples, control AAV8-GFP was injected intravenously into mice. Two weeks later, EAE was induced or not induced. The AAV control vector did not appear to interfere with development or progression of EAE (FIG. 12).

[0156] In another study, EAE was induced in C57BL/6 mice. At various times of neurological deficit of mean clinical score (MCS) ~0.3, ~0.8, or ~1.3, mice received AAV8-MOG or control vector. Mean clinical score was recorded. Even at increasing disease pathology, AAV-MOG vector had significantly reduced neurological deficit compared to control vector treated mice (FIGs. 13A-C). Bar graphs show statistical significance between final scores and peak-to-final scores.

[0157] In a further study, serial sections of spinal cord were taken from an EAE induced female mouse ~35 days after receiving control vector (MCS=4.0) . Hematoxylin and eosin stain showed areas of high inflammatory infiltration (FIG. 14A). Luxol fast blue stain showed areas of demyelination (FIG. 14B). In contrast, serial sections of spinal cord from an EAE induced female mouse ~35 days after receiving AAV-MOG vector (MCS =1.25) showed suppression of inflammation. Hematoxylin and eosin stain showed diminished infiltration (FIG. 15A). Luxol fast blue stain appeared to have less areas of demyelination as a result of the suppression of the inflammation (FIG. 15B).

[0158] In another study, regulatory T cell (Treg)-mediated suppression was measured by CFDA-SE Cell Tracer . Effector T cells (Teff) were isolated from C57BL/6 mice and labeled with CFDA. Cells were cultured either alone or in the presence of Tregs at a various Treg:Teff ratios. After 72 hours, proliferation was determined by CFDA dilution and flow cytometric analysis. Tregs isolated from spleens of AAV-MOG treated mice were found to be functionally suppressive (FIG. 16A and 16B).

[0159] In a further study, the ability of AAV-MOG to induce antigen specific Tregs was assessed. Splenocytes from mice injected with AAV-MOG vector 8 weeks prior showed an increase in frequencies of I-Ab MOG_{35-55} Tetramer positive CD4+ and Treg+ compared to control tetramer (FIGs. 17A-D), indicating that AAV-MOG vector induced antigen specific Tregs.

[0160] Next, a PLP vector was tested. AAV8-PLP was used for this part of the study. The PLP used was murine PLP. This initial proof-of-concept experiment demonstrated the timeline and clinical scoring for successful induction of PLP/EAE and the potential therapeutic benefit of liver gene transfer. Female SJL mice were injected with AAV8-PLP or control 2 weeks before immunization with 200µg PLP emulsified in CFA containing 4mg/ml Mycobacterium tuberculosis. Clinical signs of EAE began ~10 days later at which time mice were evaluated twice daily. Mice were scored according to the severity of the clinical signs (FIG. 2). Clearly, mice receiving AAV8-PLP vector had a significant reduction in disease at the peak of onset (FIG. 18). There was also a significant decrease in MCS during relapse (day 26) with fewer relapses overall (FIG. 18). These results show that AAV8-PLP reduced clinical severity in mice with PLP induced relapsing-remitting EAE. Increased reduction or complete prevention is anticipated with optimization of vector dose and timing.

[0161] Lastly, a MBP vector was tested. AAV8-MBP was used for this part of the study. The MBP used was murine MBP. Western blot analysis from protein extracted from liver of mice injected with AAV8-MBP showed an increase in MBP expression (FIG. 19A), which was consistent with an increase in mRNA levels (FIG. 19B).

[0162] It should be understood that the examples and options described herein are for illustrative purposes only Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

[0163] The description herein of any aspect or option of the disclosure using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or option of the disclosure that "consists of', "consists essentially of', or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context *(e.g.,* a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

[0164] All of the compositions and methods disclosed herein can be made and executed without undue experimentation in light of the present disclosure.

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<210> 9

<211> 247

<212> PRT

<213> Mus musculus

<400> 9

Met Ala Cys Leu Trp Ser Phe Ser Trp Pro Ser Cys Phe Leu Ser Leu
1 5 10 15

Leu Leu Leu Leu Leu Leu Gln Leu Ser Cys Ser Tyr Ala Gly Gln Phe
20 25 30

Arg Val Ile Gly Pro Gly Tyr Pro Ile Arg Ala Leu Val Gly Asp Glu
35 40 45

Ala Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly Met
50 55 60

Glu Val Gly Trp Tyr Arg Ser Pro Phe Ser Arg Val Val His Leu Tyr
65 70 75 80

Arg Asn Gly Lys Asp Gln Asp Ala Glu Gln Ala Pro Glu Tyr Arg Gly
85 90 95

Arg Thr Glu Leu Leu Lys Glu Thr Ile Ser Glu Gly Lys Val Thr Leu
100 105 110

```
Arg Ile Gln Asn Val Arg Phe Ser Asp Glu Gly Gly Tyr Thr Cys Phe
        115                 120                 125

Phe Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val
    130                 135                 140

Glu Asp Pro Phe Tyr Trp Val Asn Pro Gly Val Leu Thr Leu Ile Ala
145                 150                 155                 160

Leu Val Pro Thr Ile Leu Leu Gln Val Ser Val Gly Leu Val Phe Leu
                165                 170                 175

Phe Leu Gln His Arg Leu Arg Gly Lys Leu Arg Ala Glu Val Glu Asn
            180                 185                 190

Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp Lys
        195                 200                 205

Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala Leu
    210                 215                 220

Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu
225                 230                 235                 240

Glu Glu Leu Arg Asn Pro Phe
                245
```

<210> 10

<211> 834

<212> DNA

<213> Mus musculus

<400> 10

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atgggcttgt tagagtgttg tgctagatgt ctggtagggg ccccctttgc ttccctggtg      60

gccactggat tgtgtttctt tggagtggca ctgttctgtg gatgtggaca tgaagctctc     120

actggtacag aaaagctaat tgagacctat ttctccaaaa actaccagga ctatgagtat     180

ctcattaatg tgattcatgc tttccagtat gtcatctatg gaactgcctc tttcttcttc     240

ctttatgggg ccctcctgct ggctgagggc ttctacacca ccggcgctgt caggcagatc     300

tttggcgact acaagaccac catctgcggc aagggcctga gcgcaacggt aacaggggac     360

cagaagggga ggggttccag aggccaacat caagctcatt ctttggagcg ggtgtgtcat     420

tgtttgggaa aatggctagg acatcccgac aagtttgtgg gcatcaccta tgccctgact     480

gttgtatggc tcctggtgtt tgcctgctcg gctgtacctg tgtacattta cttcaatacc     540

tggaccacct gtcagtctat tgccttccct agcaagacct ctgccagtat aggcagtctc     600

tgcgctgatg ccagaatgta tggtgttctc ccatggaatg ctttccctgg caaggtttgt     660

ggctccaacc ttctgtccat ctgcaaaaca gctgagttcc aaatgacctt ccacctgttt     720

attgctgcgt ttgtgggtgc tgcggccaca ctagtttccc tgctcacctt catgattgct     780

gccacttaca acttcgccgt ccttaaactc atgggccgag gcaccaagtt ctga           834
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<210> 11

<211> 277

<212> PRT

<213> Mus musculus

<400> 11

```
Met Gly Leu Leu Glu Cys Cys Ala Arg Cys Leu Val Gly Ala Pro Phe
1               5                  10                  15

Ala Ser Leu Val Ala Thr Gly Leu Cys Phe Phe Gly Val Ala Leu Phe
            20                  25                  30

Cys Gly Cys Gly His Glu Ala Leu Thr Gly Thr Glu Lys Leu Ile Glu
        35                  40                  45

Thr Tyr Phe Ser Lys Asn Tyr Gln Asp Tyr Glu Tyr Leu Ile Asn Val
    50                  55                  60

Ile His Ala Phe Gln Tyr Val Ile Tyr Gly Thr Ala Ser Phe Phe Phe
65                  70                  75                  80

Leu Tyr Gly Ala Leu Leu Leu Ala Glu Gly Phe Tyr Thr Thr Gly Ala
                85                  90                  95

Val Arg Gln Ile Phe Gly Asp Tyr Lys Thr Thr Ile Cys Gly Lys Gly
            100                 105                 110

Leu Ser Ala Thr Val Thr Gly Gly Gln Lys Gly Arg Gly Ser Arg Gly
        115                 120                 125

Gln His Gln Ala His Ser Leu Glu Arg Val Cys His Cys Leu Gly Lys
    130                 135                 140

Trp Leu Gly His Pro Asp Lys Phe Val Gly Ile Thr Tyr Ala Leu Thr
145                 150                 155                 160

Val Val Trp Leu Leu Val Phe Ala Cys Ser Ala Val Pro Val Tyr Ile
                165                 170                 175

Tyr Phe Asn Thr Trp Thr Thr Cys Gln Ser Ile Ala Phe Pro Ser Lys
            180                 185                 190

Thr Ser Ala Ser Ile Gly Ser Leu Cys Ala Asp Ala Arg Met Tyr Gly
        195                 200                 205

Val Leu Pro Trp Asn Ala Phe Pro Gly Lys Val Cys Gly Ser Asn Leu
    210                 215                 220

Leu Ser Ile Cys Lys Thr Ala Glu Phe Gln Met Thr Phe His Leu Phe
225                 230                 235                 240

Ile Ala Ala Phe Val Gly Ala Ala Ala Thr Leu Val Ser Leu Leu Thr
                245                 250                 255

Phe Met Ile Ala Ala Thr Tyr Asn Phe Ala Val Leu Lys Leu Met Gly
```

Phe Met Ile Ala Ala Thr Tyr Asn Phe Ala Val Leu Lys Leu Met Gly
260 265 270

Arg Gly Thr Lys Phe
275

<210> 12

<211> 588

<212> DNA

<213> Mus musculus

<400> 12

atgggaaacc actctggaaa gagagaatta tctgctgaga aggccagtaa ggatggagag 60

attcaccgag gagaggctgg aaagaagaga agcgtgggca agctttctca gacggcctca 120

gaggacagtg atgtgtttgg ggaggcagat gcgatccaga acaatgggac ctcggctgag 180

gacacggcgg tgacagactc caagcacaca gcagacccaa agaataactg gcaaggcgcc 240

cacccagctg acccagggaa ccgccccccac ttgatccgcc tcttttcccg agatgccccg 300

ggaagggagg acaacacctt caaagacagg ccctcagagt ccgacgagct tcagaccatc 360

caagaagacc ccacagcagc ttccggaggc ctggatgtga tggcatcaca gaagagaccc 420

tcacagcgat ccaagtacct ggccacagca agtaccatgg accatgccag gcatggcttc 480

ctcccaaggc acagagacac gggcatcctt gactccatcg ggcgcttctt tagcggtgac 540

aggggtgcgc ccaagcgggg ctctggcaag gtgagctccg agccgtag 588

<210> 13

<211> 195

<212> PRT

<213> Mus musculus

<400> 13

Met Gly Asn His Ser Gly Lys Arg Glu Leu Ser Ala Glu Lys Ala Ser
1 5 10 15

Lys Asp Gly Glu Ile His Arg Gly Glu Ala Gly Lys Lys Arg Ser Val
20 25 30

Gly Lys Leu Ser Gln Thr Ala Ser Glu Asp Ser Asp Val Phe Gly Glu
35 40 45

Ala Asp Ala Ile Gln Asn Asn Gly Thr Ser Ala Glu Asp Thr Ala Val
50 55 60

Thr Asp Ser Lys His Thr Ala Asp Pro Lys Asn Asn Trp Gln Gly Ala
65 70 75 80

His Pro Ala Asp Pro Gly Asn Arg Pro His Leu Ile Arg Leu Phe Ser
85 90 95

Arg Asp Ala Pro Gly Arg Glu Asp Asn Thr Phe Lys Asp Arg Pro Ser
100 105 110

```
Glu Ser Asp Glu Leu Gln Thr Ile Gln Glu Asp Pro Thr Ala Ala Ser
        115                 120                 125

Gly Gly Leu Asp Val Met Ala Ser Gln Lys Arg Pro Ser Gln Arg Ser
    130                 135                 140

Lys Tyr Leu Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe
145                 150                 155                 160

Leu Pro Arg His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe
                165                 170                 175

Phe Ser Gly Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Val Ser
            180                 185                 190

Ser Glu Pro
        195
```

<210> 14

<211> 744

<212> DNA

<213> Homo sapiens

<400> 14

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atggcaagct tatcgagacc ctctctgccc agctgcctct gctccttcct cctcctcctc       60

ctcctccaag tgtcttccag ctatgcaggg cagttcagag tgataggacc aagacaccct      120

atccgggctc tggtcgggga tgaagtggaa ttgccatgtc gcatatctcc tgggaagaac      180

gctacaggca tggaggtggg gtggtaccgc ccccccttct ctagggtggt tcatctctac      240

agaaatggca aggaccaaga tggagaccag gcacctgaat atcggggccg gacagagctg      300

ctgaaagatg ctattggtga gggaaaggtg actctcagga tccggaatgt aaggttctca      360

gatgaaggag gtttcacctg cttcttccga gatcattctt accaagagga ggcagcaatg      420

gaattgaaag tagaagatcc tttctactgg gtgagccctg gagtgctggt tctcctcgcg      480

gtgctgcctg tgctcctcct gcagatcact gttggcctcg tcttcctctg cctgcagtac      540

agactgagag gaaaacttcg agcagagata gagaatctcc accggacttt tgatccccac      600

tttctgaggg tgccctgctg gaagataacc ctgtttgtaa ttgtgccggt tcttggaccc      660

ttggttgcct tgatcatctg ctacaactgg ctacatcgaa gactagcagg gcaattcctt      720

gaagagctac gaaatccctt ctga                                             744
```

<210> 15

<211> 247

<212> PRT

<213> Homo sapiens

<400> 15

```
Met Ala Ser Leu Ser Arg Pro Ser Leu Pro Ser Cys Leu Cys Ser Phe
1               5                   10                  15
```

```
Leu Leu Leu Leu Leu Leu Gln Val Ser Ser Ser Tyr Ala Gly Gln Phe
            20                  25                  30

Arg Val Ile Gly Pro Arg His Pro Ile Arg Ala Leu Val Gly Asp Glu
        35                  40                  45

Val Glu Leu Pro Cys Arg Ile Ser Pro Gly Lys Asn Ala Thr Gly Met
    50                  55                  60

Glu Val Gly Trp Tyr Arg Pro Pro Phe Ser Arg Val Val His Leu Tyr
65                  70                  75                  80

Arg Asn Gly Lys Asp Gln Asp Gly Asp Gln Ala Pro Glu Tyr Arg Gly
                85                  90                  95

Arg Thr Glu Leu Leu Lys Asp Ala Ile Gly Glu Gly Lys Val Thr Leu
            100                 105                 110

Arg Ile Arg Asn Val Arg Phe Ser Asp Glu Gly Gly Phe Thr Cys Phe
        115                 120                 125

Phe Arg Asp His Ser Tyr Gln Glu Glu Ala Ala Met Glu Leu Lys Val
    130                 135                 140

Glu Asp Pro Phe Tyr Trp Val Ser Pro Gly Val Leu Val Leu Leu Ala
145                 150                 155                 160

Val Leu Pro Val Leu Leu Leu Gln Ile Thr Val Gly Leu Val Phe Leu
                165                 170                 175

Cys Leu Gln Tyr Arg Leu Arg Gly Lys Leu Arg Ala Glu Ile Glu Asn
            180                 185                 190

Leu His Arg Thr Phe Asp Pro His Phe Leu Arg Val Pro Cys Trp Lys
        195                 200                 205

Ile Thr Leu Phe Val Ile Val Pro Val Leu Gly Pro Leu Val Ala Leu
    210                 215                 220

Ile Ile Cys Tyr Asn Trp Leu His Arg Arg Leu Ala Gly Gln Phe Leu
225                 230                 235                 240

Glu Glu Leu Arg Asn Pro Phe
                245
```

<210> 16

<211> 915

<212> DNA

<213> Homo sapiens

<400> 16

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atgggaaacc acgcaggcaa acgagaatta aatgccgaga aggccagtac gaatagtgaa        60
```

actaacagag gagaatctga aaaaaagaga aacctgggtg aactttcacg gacaacctca 120

gaggacaacg aagtgttcgg agaggcagat gcgaaccaga acaatgggac ctcctctcag 180

gacacagcgg tgactgactc caagcgcaca gcggacccga agaatgcctg gcaggatgcc 240

cacccagctg acccagggag ccgcccccac ttgatccgcc tcttttcccg agatgccccg 300

gggagggagg acaacacctt caaagacagg ccctctgagt ccgacgagct ccagaccatc 360

caagaagaca gtgcagccac ctccgagagc ctggatgtga tggcgtcaca gaagagaccc 420

tcccagaggc acggatccaa gtacctggcc acagcaagta ccatggacca tgccaggcat 480

ggcttcctcc caaggcacag agacacgggc atccttgact ccatcgggcg cttctttggc 540

ggtgacaggg gtgcgcccaa gcggggctct ggcaaggact cacaccaccc ggcaagaact 600

gctcactacg gctccctgcc ccagaagtca cacggccgga cccaagatga aaaccccgta 660

gtccacttct tcaagaacat tgtgacgcct cgcacaccac ccccgtcgca gggaaagggg 720

agaggactgt ccctgagcag atttagctgg ggggccgaag gccagagacc aggatttggc 780

tacggaggca gagcgtccga ctataaatcg gctcacaagg gattcaaggg agtcgatgcc 840

cagggcacgc tttccaaaat ttttaagctg ggaggaagag atagtcgctc tggatcaccc 900

atggctagac gctga 915

<210> 17

<211> 304

<212> PRT

<213> Homo sapiens

<400> 17

```
Met Gly Asn His Ala Gly Lys Arg Glu Leu Asn Ala Glu Lys Ala Ser
1               5                  10                  15

Thr Asn Ser Glu Thr Asn Arg Gly Glu Ser Glu Lys Lys Arg Asn Leu
            20                  25                  30

Gly Glu Leu Ser Arg Thr Thr Ser Glu Asp Asn Glu Val Phe Gly Glu
        35                  40                  45

Ala Asp Ala Asn Gln Asn Asn Gly Thr Ser Ser Gln Asp Thr Ala Val
    50                  55                  60

Thr Asp Ser Lys Arg Thr Ala Asp Pro Lys Asn Ala Trp Gln Asp Ala
65                  70                  75                  80

His Pro Ala Asp Pro Gly Ser Arg Pro His Leu Ile Arg Leu Phe Ser
                85                  90                  95

Arg Asp Ala Pro Gly Arg Glu Asp Asn Thr Phe Lys Asp Arg Pro Ser
            100                 105                 110

Glu Ser Asp Glu Leu Gln Thr Ile Gln Glu Asp Ser Ala Ala Thr Ser
```

```
        115                 120                 125

Glu Ser Leu Asp Val Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His
    130                 135                 140

Gly Ser Lys Tyr Leu Ala Thr Ala Ser Thr Met Asp His Ala Arg His
145                 150                 155                 160

Gly Phe Leu Pro Arg His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly
                165                 170                 175

Arg Phe Phe Gly Gly Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys
            180                 185                 190

Asp Ser His His Pro Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln
        195                 200                 205

Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro Val Val His Phe Phe
    210                 215                 220

Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly
225                 230                 235                 240

Arg Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Arg
                245                 250                 255

Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser Ala His
            260                 265                 270

Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe
        275                 280                 285

Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
    290                 295                 300
```

<210> 18

<211> 594

<212> DNA

<213> Homo sapiens

<400> 18

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atggcgtcac agaagagacc ctcccagagg cacggatcca agtacctggc cacagcaagt      60

accatggacc atgccaggca tggcttcctc ccaaggcaca gagacacggg catccttgac     120

tccatcgggc gcttctttgg cggtgacagg ggtgcgccca agcggggctc tggcaaggta     180

ccctggctaa agccgggccg gagccctctg ccctctcatg cccgcagcca gcctgggctg     240

tgcaacatgt acaaggactc acaccacccg gcaagaactg ctcactacgg ctccctgccc     300

cagaagtcac acggccggac ccaagatgaa aaccccgtag tccacttctt caagaacatt     360

gtgacgcctc gcacaccacc cccgtcgcag ggaaagggga gaggactgtc cctgagcaga     420

tttagctggg gggccgaagg ccagagacca ggatttggct acggaggcag agcgtccgac     480
```

tataaatcgg ctcacaaggg attcaaggga gtcgatgccc agggcacgct ttccaaaatt 540

tttaagctgg gaggaagaga tagtcgctct ggatcaccca tggctagacg ctga 594

<210> 19

<211> 197

<212> PRT

<213> Homo sapiens

<400> 19

Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1 5 10 15

Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
20 25 30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
35 40 45

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Val Pro Trp Leu Lys
50 55 60

Pro Gly Arg Ser Pro Leu Pro Ser His Ala Arg Ser Gln Pro Gly Leu
65 70 75 80

Cys Asn Met Tyr Lys Asp Ser His His Pro Ala Arg Thr Ala His Tyr
85 90 95

Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro
100 105 110

Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro
115 120 125

Ser Gln Gly Lys Gly Arg Gly Leu Ser Leu Ser Arg Phe Ser Trp Gly
130 135 140

Ala Glu Gly Gln Arg Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp
145 150 155 160

Tyr Lys Ser Ala His Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr
165 170 175

Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser
180 185 190

Pro Met Ala Arg Arg
195

<210> 20

<211> 561

<212> DNA

<213> Homo sapiens

<400> 20

atggcgtcac agaagagacc ctcccagagg cacggatcca agtacctggc cacagcaagt 60

accatggacc atgccaggca tggcttcctc ccaaggcaca gagacacggg catccttgac 120

tccatcgggc gcttctttgg cggtgacagg ggtgcgccca agcggggctc tggcaaggta 180

ccctggctaa agccgggccg gagccctctg ccctctcatg cccgcagcca gcctgggctg 240

tgcaacatgt acaaggactc acaccacccg gcaagaactg ctcactacgg ctccctgccc 300

cagaagtcac acggccggac ccaagatgaa aaccccgtag tccacttctt caagaacatt 360

gtgacgcctc gcacaccacc cccgtcgcag ggaaaggggg ccgaaggcca gagaccagga 420

tttggctacg gaggcagagc gtccgactat aaatcggctc acaagggatt caagggagtc 480

gatgcccagg gcacgctttc caaaattttt aagctgggag gaagagatag tcgctctgga 540

tcacccatgg ctagacgctg a 561

<210> 21

<211> 186

<212> PRT

<213> Homo sapiens

<400> 21

Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1 5 10 15

Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
20 25 30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
35 40 45

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Val Pro Trp Leu Lys
50 55 60

Pro Gly Arg Ser Pro Leu Pro Ser His Ala Arg Ser Gln Pro Gly Leu
65 70 75 80

Cys Asn Met Tyr Lys Asp Ser His His Pro Ala Arg Thr Ala His Tyr
85 90 95

Gly Ser Leu Pro Gln Lys Ser His Gly Arg Thr Gln Asp Glu Asn Pro
100 105 110

Val Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro Pro
115 120 125

Ser Gln Gly Lys Gly Ala Glu Gly Gln Arg Pro Gly Phe Gly Tyr Gly
130 135 140

Gly Arg Ala Ser Asp Tyr Lys Ser Ala His Lys Gly Phe Lys Gly Val
145 150 155 160

```
Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg Asp
                165                 170                 175

Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
            180                 185
```

<210> 22

<211> 516

<212> DNA

<213> Homo sapiens

<400> 22

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atggcgtcac agaagagacc ctcccagagg cacggatcca agtacctggc cacagcaagt      60
accatggacc atgccaggca tggcttcctc ccaaggcaca gagacacggg catccttgac     120
tccatcgggc gcttctttgg cggtgacagg ggtgcgccca agcggggctc tggcaaggac     180
tcacaccacc cggcaagaac tgctcactac ggctccctgc cccagaagtc acacggccgg     240
acccaagatg aaaaccccgt agtccacttc ttcaagaaca ttgtgacgcc tcgcacacca     300
cccccgtcgc agggaaaggg gagaggactg tccctgagca gatttagctg gggggccgaa     360
ggccagagac caggatttgg ctacggaggc agagcgtccg actataaatc ggctcacaag     420
ggattcaagg gagtcgatgc ccagggcacg ctttccaaaa tttttaagct gggaggaaga     480
gatagtcgct ctggatcacc catggctaga cgctga                               516
```

<210> 23

<211> 171

<212> PRT

<213> Homo sapiens

<400> 23

```
Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1               5                   10                  15

Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
            20                  25                  30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
        35                  40                  45

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Asp Ser His His Pro
    50                  55                  60

Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg
65                  70                  75                  80

Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr
                85                  90                  95

Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly Arg Gly Leu Ser Leu
            100                 105                 110
```

100 105 110

Ser Arg Phe Ser Trp Gly Ala Glu Gly Gln Arg Pro Gly Phe Gly Tyr
115 120 125

Gly Gly Arg Ala Ser Asp Tyr Lys Ser Ala His Lys Gly Phe Lys Gly
130 135 140

Val Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe Lys Leu Gly Gly Arg
145 150 155 160

Asp Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
165 170

<210> 24

<211> 483

<212> DNA

<213> Homo sapiens

<400> 24

atggcgtcac agaagagacc ctcccagagg cacggatcca agtacctggc cacagcaagt 60

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tccatcgggc gcttctttgg cggtgacagg ggtgcgccca agcggggctc tggcaaggac 180

tcacaccacc cggcaagaac tgctcactac ggctccctgc cccagaagtc acacggccgg 240

acccaagatg aaaaccccgt agtccacttc ttcaagaaca ttgtgacgcc tcgcacacca 300

cccccgtcgc agggaaaggg ggccgaaggc cagagaccag gatttggcta cggaggcaga 360

gcgtccgact ataaatcggc tcacaaggga ttcaagggag tcgatgccca gggcacgctt 420

tccaaaattt ttaagctggg aggaagagat agtcgctctg gatcacccat ggctagacgc 480

tga 483

<210> 25

<211> 160

<212> PRT

<213> Homo sapiens

<400> 25

Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His Gly Ser Lys Tyr Leu
1 5 10 15

Ala Thr Ala Ser Thr Met Asp His Ala Arg His Gly Phe Leu Pro Arg
20 25 30

His Arg Asp Thr Gly Ile Leu Asp Ser Ile Gly Arg Phe Phe Gly Gly
35 40 45

Asp Arg Gly Ala Pro Lys Arg Gly Ser Gly Lys Asp Ser His His Pro
50 55 60

Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg

Ala Arg Thr Ala His Tyr Gly Ser Leu Pro Gln Lys Ser His Gly Arg
65 70 75 80

Thr Gln Asp Glu Asn Pro Val Val His Phe Phe Lys Asn Ile Val Thr
85 90 95

Pro Arg Thr Pro Pro Pro Ser Gln Gly Lys Gly Ala Glu Gly Gln Arg
100 105 110

Pro Gly Phe Gly Tyr Gly Gly Arg Ala Ser Asp Tyr Lys Ser Ala His
115 120 125

Lys Gly Phe Lys Gly Val Asp Ala Gln Gly Thr Leu Ser Lys Ile Phe
130 135 140

Lys Leu Gly Gly Arg Asp Ser Arg Ser Gly Ser Pro Met Ala Arg Arg
145 150 155 160

<210> 26

<211> 594

<212> DNA

<213> Homo sapiens

<400> 26

atgggaaacc acgcaggcaa acgagaatta aatgccgaga aggccagtac gaatagtgaa 60

actaacagag gagaatctga aaaaaagaga aacctgggtg aactttcacg gacaacctca 120

gaggacaacg aagtgttcgg agaggcagat gcgaaccaga acaatgggac ctcctctcag 180

gacacagcgg tgactgactc caagcgcaca gcggacccga agaatgcctg gcaggatgcc 240

cacccagctg acccagggag ccgccccccac ttgatccgcc tcttttcccg agatgccccg 300

gggagggagg acaacacctt caaagacagg ccctctgagt ccgacgagct ccagaccatc 360

caagaagaca gtgcagccac ctccgagagc ctggatgtga tggcgtcaca gaagagaccc 420

tcccagaggc acggatccaa gtacctggcc acagcaagta ccatggacca tgccaggcat 480

ggcttcctcc caaggcacag agacacgggc atccttgact ccatcgggcg cttctttggc 540

ggtgacaggg gtgcgcccaa gcggggctct ggcaaggtga gctctgagga gtag 594

<210> 27

<211> 197

<212> PRT

<213> Homo sapiens

<400> 27

Met Gly Asn His Ala Gly Lys Arg Glu Leu Asn Ala Glu Lys Ala Ser
1 5 10 15

Thr Asn Ser Glu Thr Asn Arg Gly Glu Ser Glu Lys Lys Arg Asn Leu
20 25 30

Gly Glu Leu Ser Arg Thr Thr Ser Glu Asp Asn Glu Val Phe Gly Glu
35 40 45

```
Ala Asp Ala Asn Gln Asn Asn Gly Thr Ser Ser Gln Asp Thr Ala Val
    50                  55                  60

Thr Asp Ser Lys Arg Thr Ala Asp Pro Lys Asn Ala Trp Gln Asp Ala
65                  70                  75                  80

His Pro Ala Asp Pro Gly Ser Arg Pro His Leu Ile Arg Leu Phe Ser
                85                  90                  95

Arg Asp Ala Pro Gly Arg Glu Asp Asn Thr Phe Lys Asp Arg Pro Ser
            100                 105                 110

Glu Ser Asp Glu Leu Gln Thr Ile Gln Glu Asp Ser Ala Ala Thr Ser
        115                 120                 125

Glu Ser Leu Asp Val Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His
    130                 135                 140

Gly Ser Lys Tyr Leu Ala Thr Ala Ser Thr Met Asp His Ala Arg His
145                 150                 155                 160

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Trp Asn Ala Phe Pro Gly Lys Val Cys Gly Ser Asn Leu Leu Ser Ile
            180                 185                 190

Cys Lys Thr Ala Glu Phe Gln Met Thr Phe His Leu Phe Ile Ala Ala
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Phe Val Gly Ala Ala Ala Thr Leu Val Ser Leu Leu Thr Phe Met Ile
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Val Arg Gln Ile Phe Gly Asp Tyr Lys Thr Thr Ile Cys Gly Lys Gly
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Phe Val Gly Ile Thr Tyr Ala Leu Thr Val Val Trp Leu Leu Val Phe
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Cys Gln Ser Ile Ala Phe Pro Ser Lys Thr Ser Ala Ser Ile Gly Ser
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Pro Gly Lys Val Cys Gly Ser Asn Leu Leu Ser Ile Cys Lys Thr Ala
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Glu Phe Gln Met Thr Phe His Leu Phe Ile Ala Ala Phe Val Gly Ala
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REFERENCES CITED IN THE DESCRIPTION

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AVV-BASERET GENTERAPI TIL MULTIPEL SCLEROSE

Patentkrav

1. En rekombinant adeno-associeret viral (rAAV) nukleinsyrevektor fra AAV8-serotypen, som omfatter:
en polynukleotid, der omfatter et nukleinsyresegment, der koder et myelin-basisprotein (MBP) fra pattedyr i fuld længde, proteolipidprotein (PLP) eller myelin-oligodendrocytglykoprotein (MOG), der er funktionsmæssigt kædet til en promotor, der er i stand til at udtrykke nukleinsyresegmentet i én eller flere celler i en pattedyrslever.

2. rAAV-nukleinsyrevektoren i krav 1, hvori det MBP, PLP eller MOG er af human oprindelse.

3. rAAV-nukleinsyrevektor i krav 1, hvori nukleinsyresegmentet koder et myelin-basisprotein (MBP), proteolipidprotein (PLP) eller myelin-oligodendrocytglykoprotein (MOG), som omfatter en aminosyresekvens, der er mindst 95 % identisk med sekvensen SEQ ID NO:1, SEQ ID NO:2 eller SEQ ID NO:3.

4. rAAV-nukleinsyrevektoren i ethvert af kravene 1 til 3, hvori nukleinsyresegmentet yderligere omfatter en forstærker, en post-transkriptionsreguleringssekvens, et polyadenyleringssignal eller enhver kombination deraf, som er funktionsmæssigt kædet til et nukleinsyremolesegment, der koder det terapeutiske molekyle.

5. rAAV-nukleinsyrevektor i ethvert af kravene 1 til 4, hvori promotoren er en pattedyrscellespecifik eller en pattedyrsvævsspecifik promotor.

6. rAAV-nukleinsyrevektoren i ethvert af kravene 1 til 5, hvori nukleinsyresegmentet desuden koder eller yderligere udtrykker et polypeptid, et peptid, et ribozym, en peptidnukleinsyre, en siRNA, en RNAi, en antisense oligonukleotid, en antisense oligonukleotid, en antisense polynukleotid, et antistof, et antigenbindende fragment eller enhver kombination deraf.

7. En rAAV-partikel, der omfatter rAAV-nukleinsyrevektoren for ethvert af kravene 1 til 6.

8. rAAV-nukleinsyrevektoren i ethvert af kravene 1 til 6 eller rAAV-partiklen i krav 7 til brug i lægemiddel.

9. rAAV-nukleinsyrevektoren i ethvert af kravene 1 til 6 eller rAAV-partiklen i krav 7 til brug i behandlingen eller forbedring af ét eller flere symptomer på en autoimmunsygdom i et pattedyr, hvori rAAV-nukleinsyrevektoren eller rAAV-partiklen indgives systemisk til pattedyret i en mængde og et tidsrum, som er tilstrækkeligt til at behandle eller forbedre det ene eller flere symptomer på autoimmunsygdom i pattedyret, hvori autoimmunsygdommen er multipel sclerose, dissemineret sclerose eller dissemineret encephalomyelitis.

10. rAAV-nukleinsyrevektoren eller rAAV-partiklen til brug i henhold til krav 9, hvori pattedyret er en nyfødt, et spædbarn, et barn eller en ung voksen.

11. rAAV-nukleinsyrevektoren eller rAAV-partiklen til brug i henhold til krav 9 eller krav 10, hvori produktionen af det terapeutiske molekyle i pattedyret reducerer CNS-inflammation, hæmmer demyelinisation, gendanner immuntolerancen over for ét eller flere neuroproteiner, stimulerer produktionen af endogene antigenspecifikke regulatoriske T-celler eller enhver kombination deraf.

12. rAAV-nukleinsyrevektoren eller rAAV-partiklen til brug i henhold til ethvert af krav 9 til 11, hvori rAAV-vektoren desuden koder en agonist, an antagonist, en anti-apoptosefaktor, en inhibitor, en receptor, et cytokin, et cytotoksin, et erythropoietisk middel, et glykoprotein, en vækstfaktor, en vækstfaktorreceptor, et hormon, en hormonreceptor, en interferon, en interleukin, en interleukinreceptor, en nervevækstfaktor, et neuroaktivt peptid, en neuroaktiv peptidreceptor, en protease, en proteaseinhibitor, en protein-decarboxylase, en proteinkinase, en proteinkinaseinhibitor, et enzym, et receptorbindende protein, et transportprotein eller en inhibitor deraf, en serotoninreceptor eller en optagelsesinhibitor deraf, en serpin, en serpinreceptor, en tumorsuppressor, et kemoterapeutisk middel eller enhver kombination deraf.

13. rAAV-nukleinsyrevektor i krav 2, hvori nukleinsyresegmentet koder et myelin-basisprotein (MBP), proteolipidprotein (PLP) eller myelin-oligodendrocytglykoprotein

(MOG), som omfatter en aminosyresekvens, der er mindst 95 % identisk med sekvensen SEQ ID NO:17, SEQ ID NO:29 eller SEQ ID NO:15.

14. rAAV-nukleinsyrevektor i krav 2, hvori nukleinsyresegmentet koder et myelin-basisprotein (MBP), proteolipidprotein (PLP) eller myelin-oligodendrocytglykoprotein (MOG), der omfatter en aminosyresekvens, som angivet i SEQ ID NO:17, SEQ ID NO:29 eller SEQ ID NO:15.

DRAWINGS

Used to study pathogenesis of autoimmunity, CNS inflammation, demyelination, cell trafficking and tolerance induction.

Characterized by paralysis, CNS inflammation and demyelination, mediated by myelin-specific CD4+ T cells, but CD8+ cells and B cells also play a role in some models of EAE.

Several neuro proteins or peptides are used to induce EAE including:

Myelin Basic Protein (MBP)
Proteolipid Protein (PLP)
Myelin Oligodendrocyte Glycoprotein (MOG)

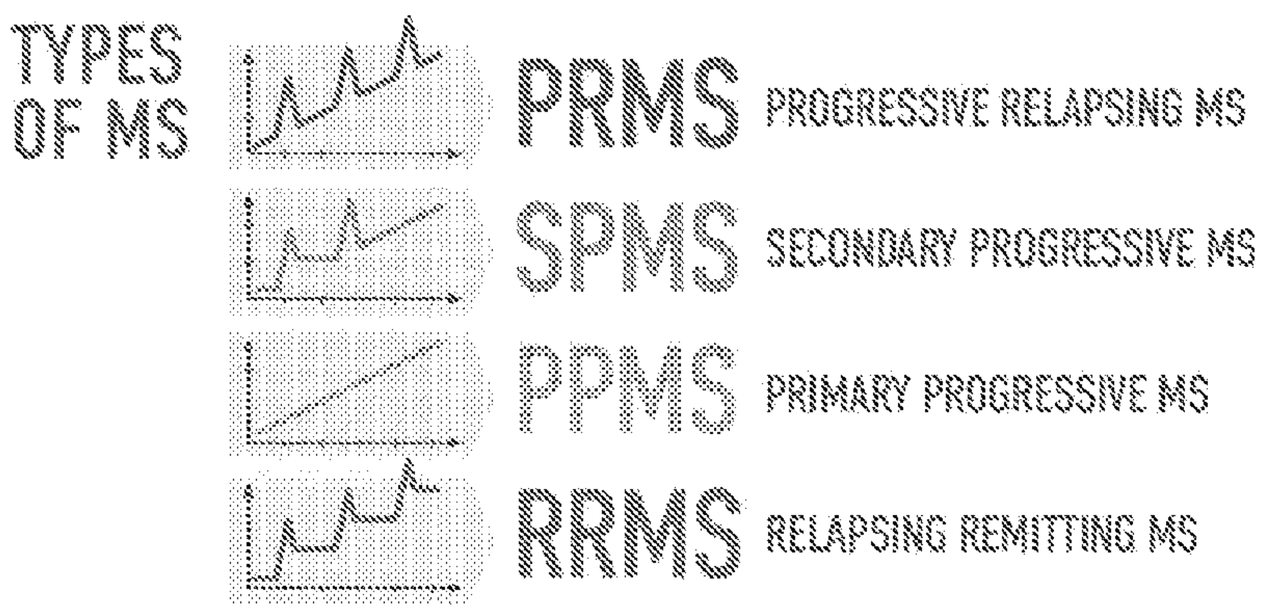


FIG. 1

Peptide/CFA

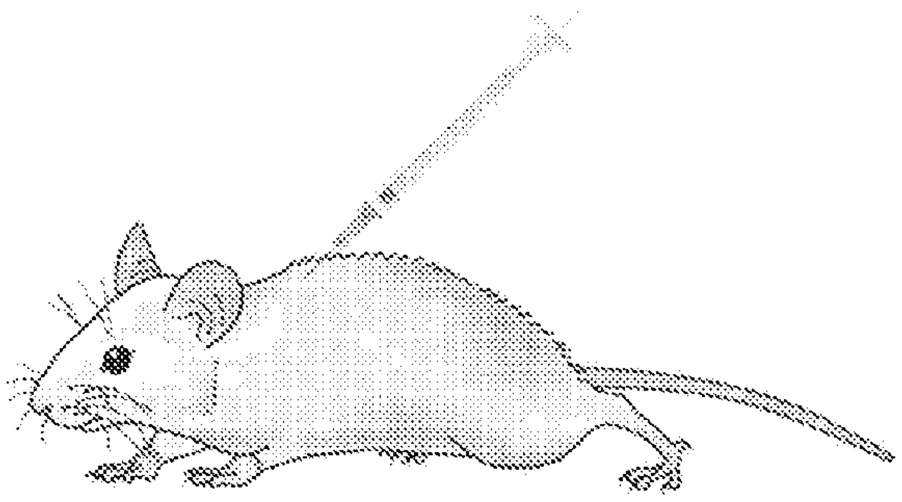


FIG. 2A

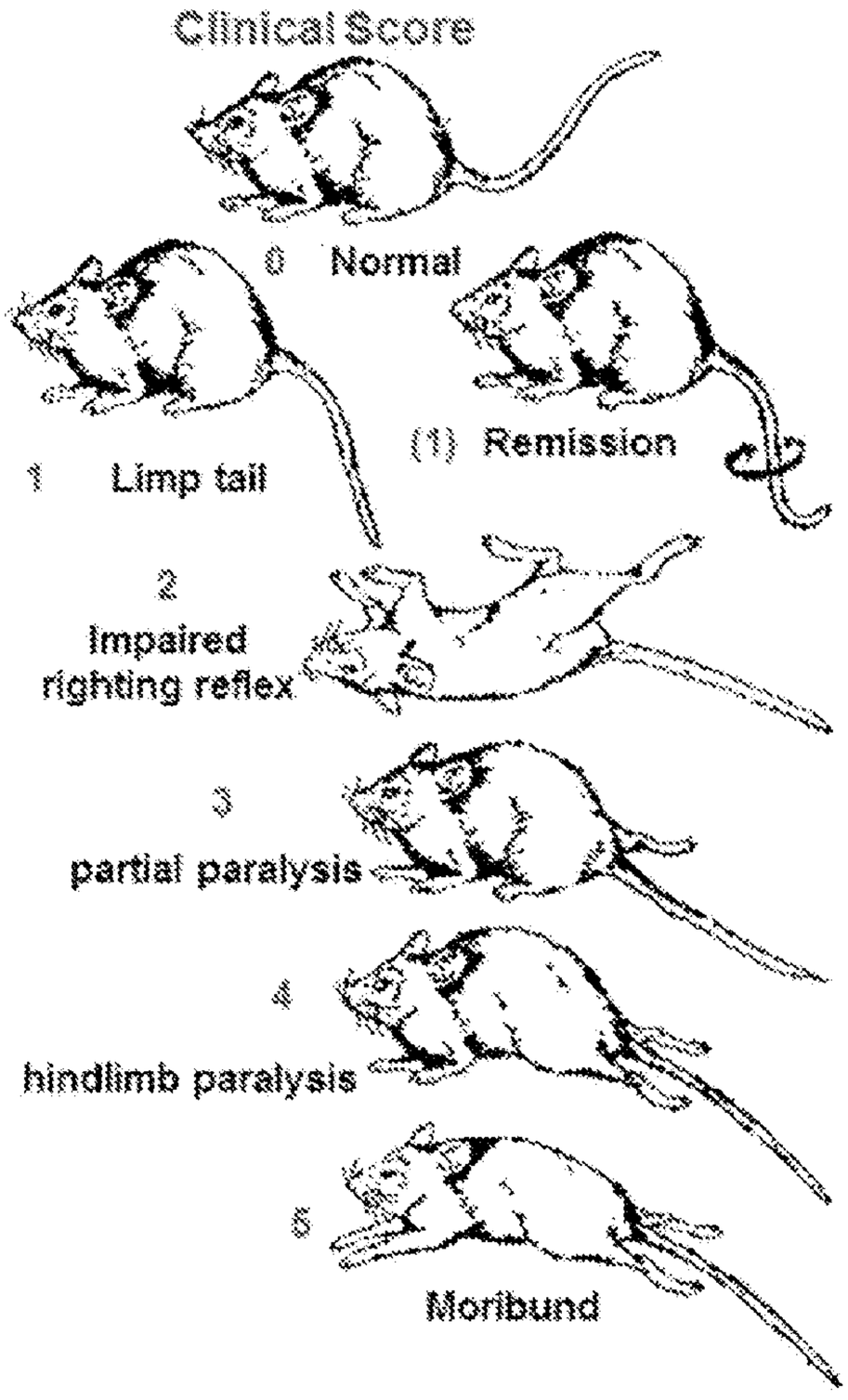

Clinical Score
0 Normal
(1) Remission
1 Limp tail
2
Impaired
righting reflex
3
partial paralysis
4
hindlimb paralysis
5
Moribund

FIG. 2B

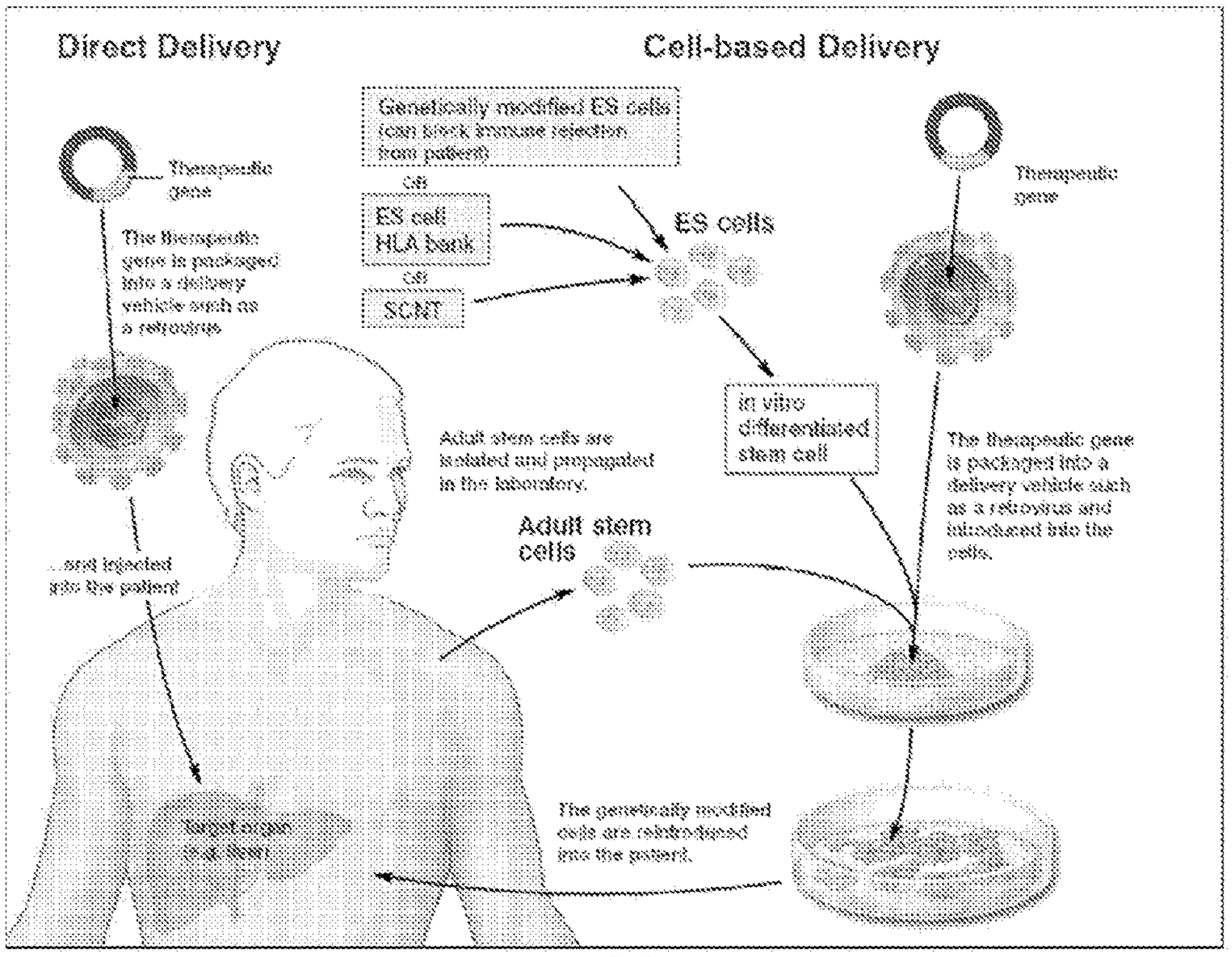


FIG. 3

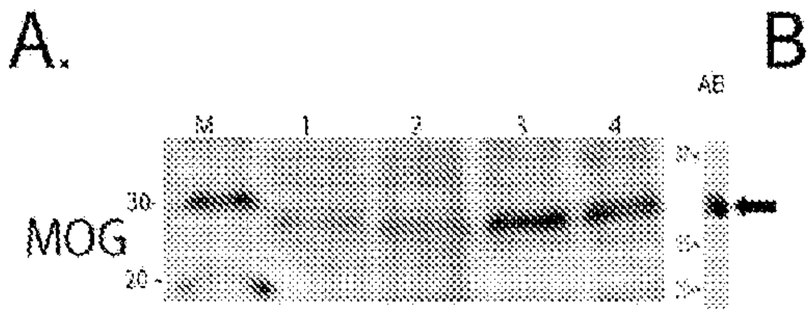


FIG. 4A

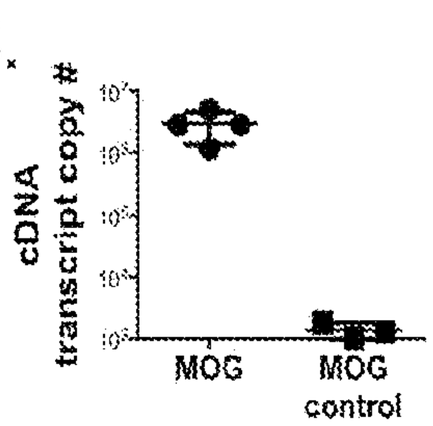


FIG. 4B

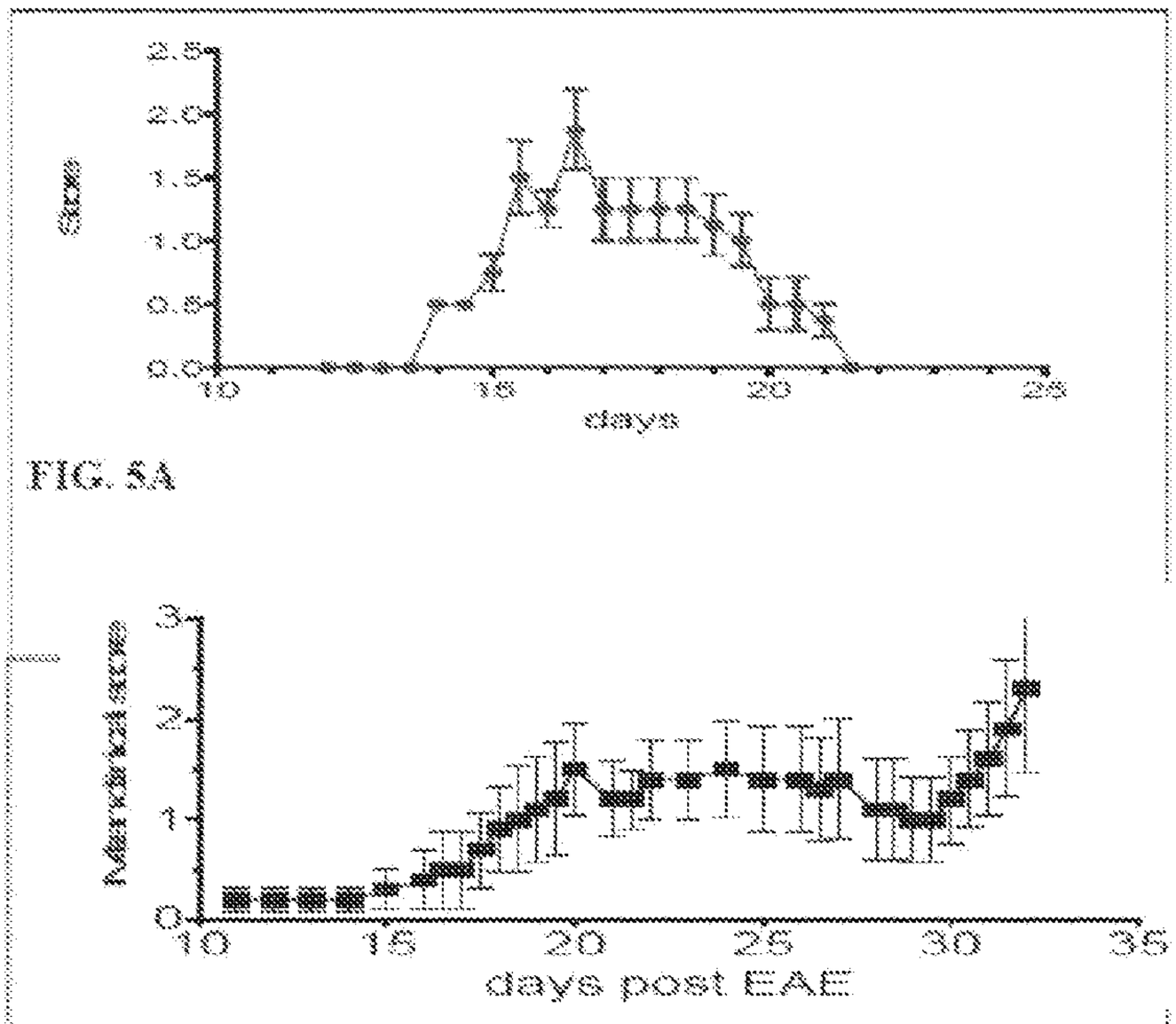

2.5
2.0
1.5
1.0
0.5
0.0
10
15
20
25
days

FIG. 5A

Mean clinical score
3
2
1
0
10
15
20
25
30
35
days post EAE

FIG. 5B

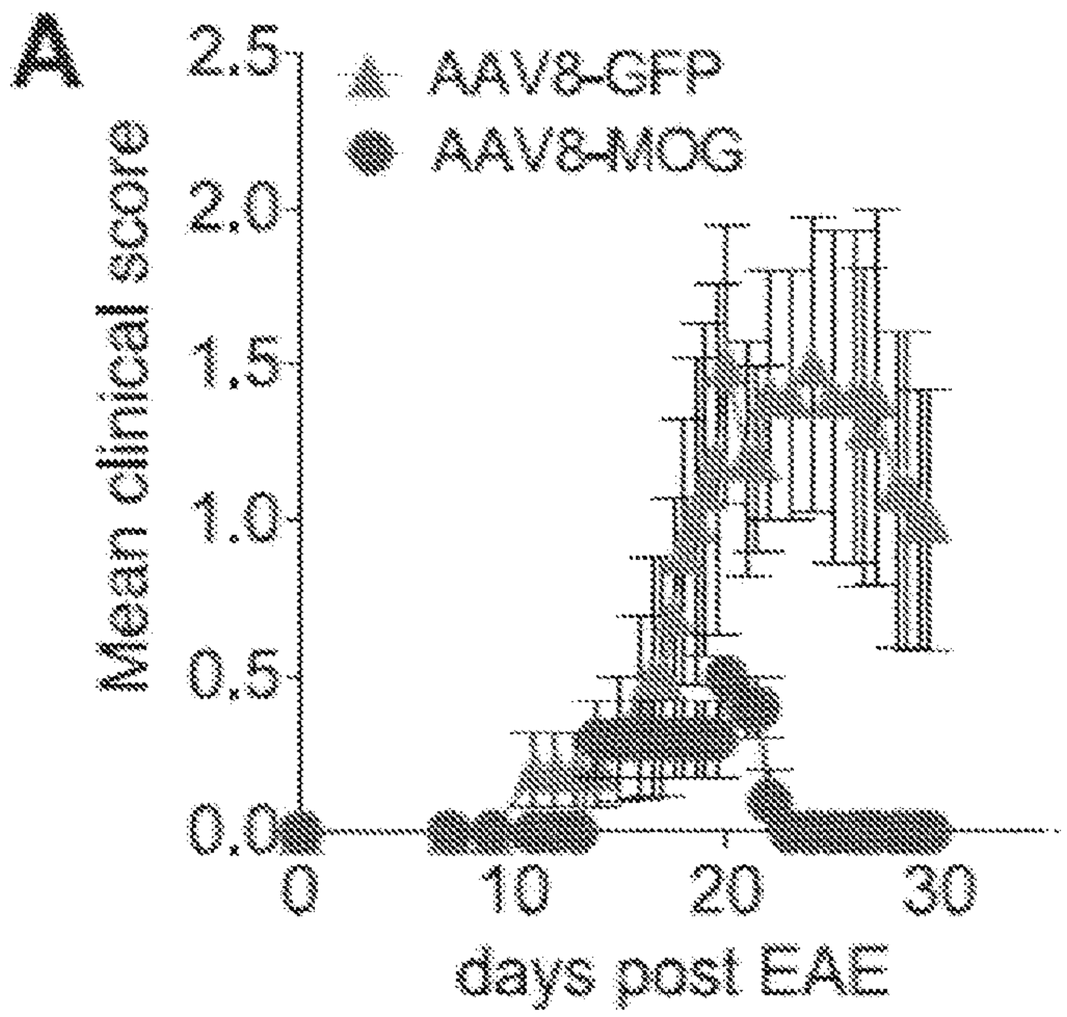

A
AAV8-GFP
AAV8-MOG
Mean clinical score
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
days post EAE

FIG. 6A

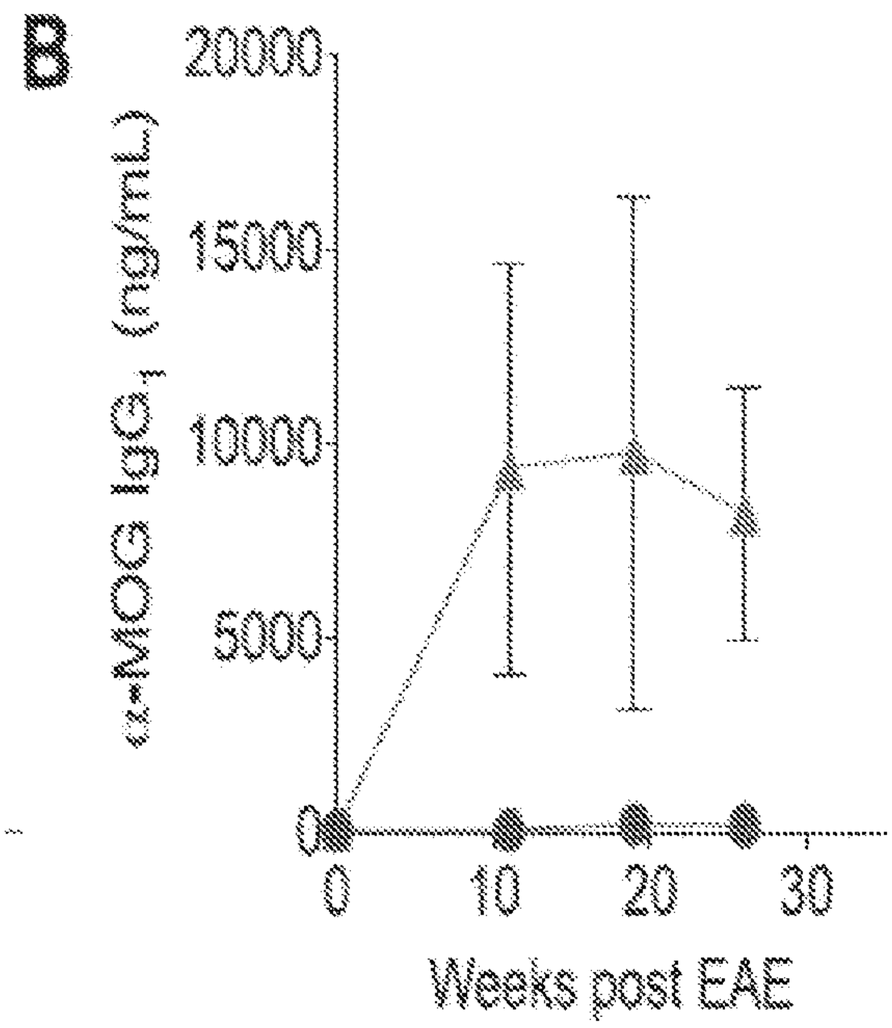

B
anti-MOG IgG (ng/mL)
20000
15000
10000
5000
0
0
10
20
30
Weeks post EAE

FIG. 6B

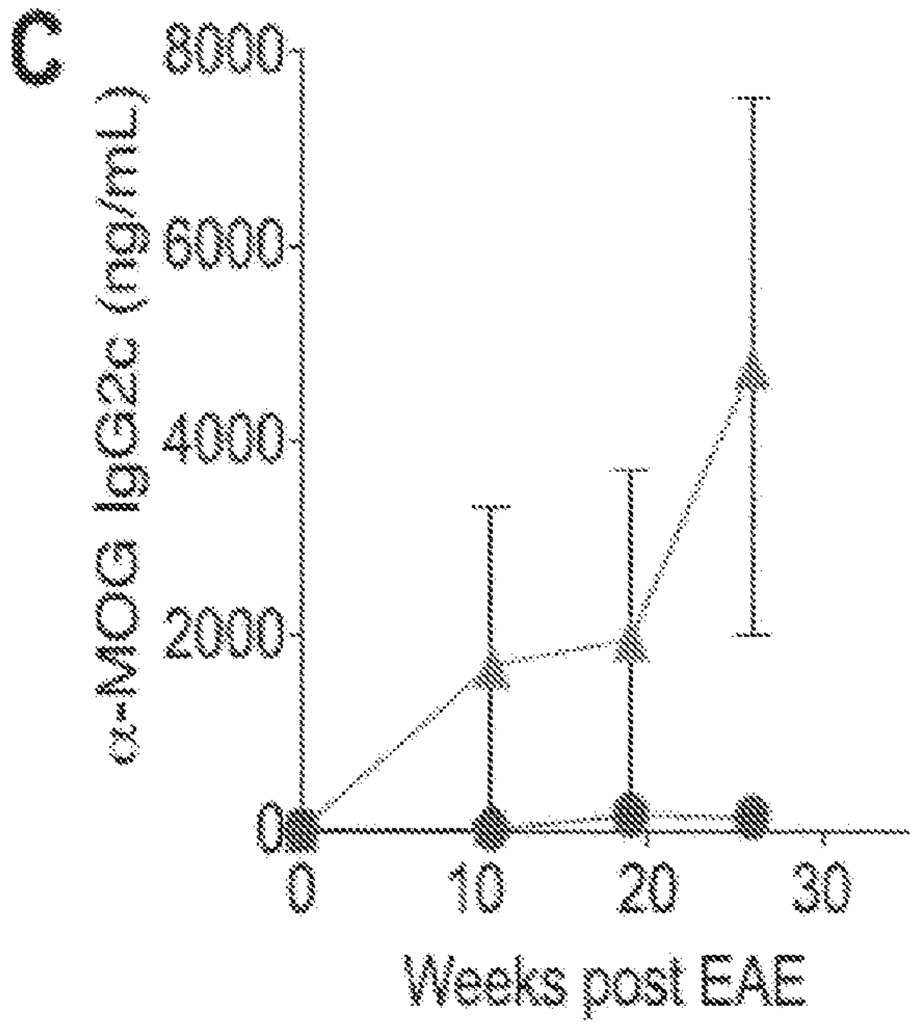

C
α-MOG IgG2c (ng/mL)
8000
6000
4000
2000
0
0
10
20
30
Weeks post EAE

FIG. 6C

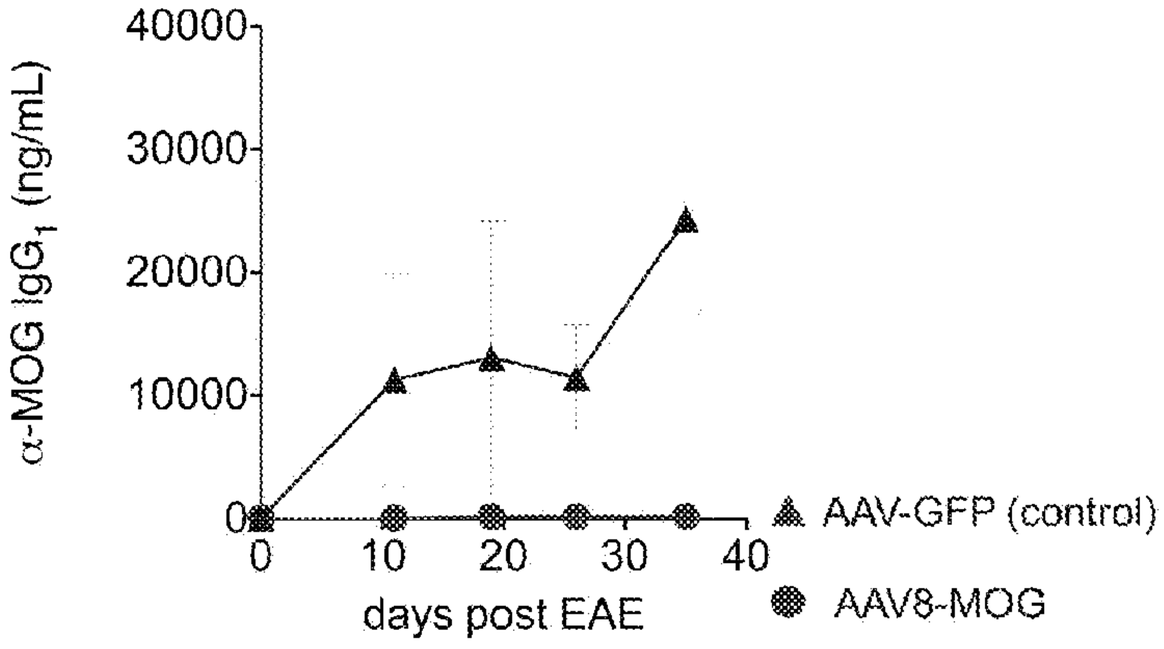

α-MOG IgG1 (ng/mL)
40000
30000
20000
10000
0
0
10
20
30
40
days post EAE
AAV-GFP (control)
AAV8-MOG

FIG. 7A

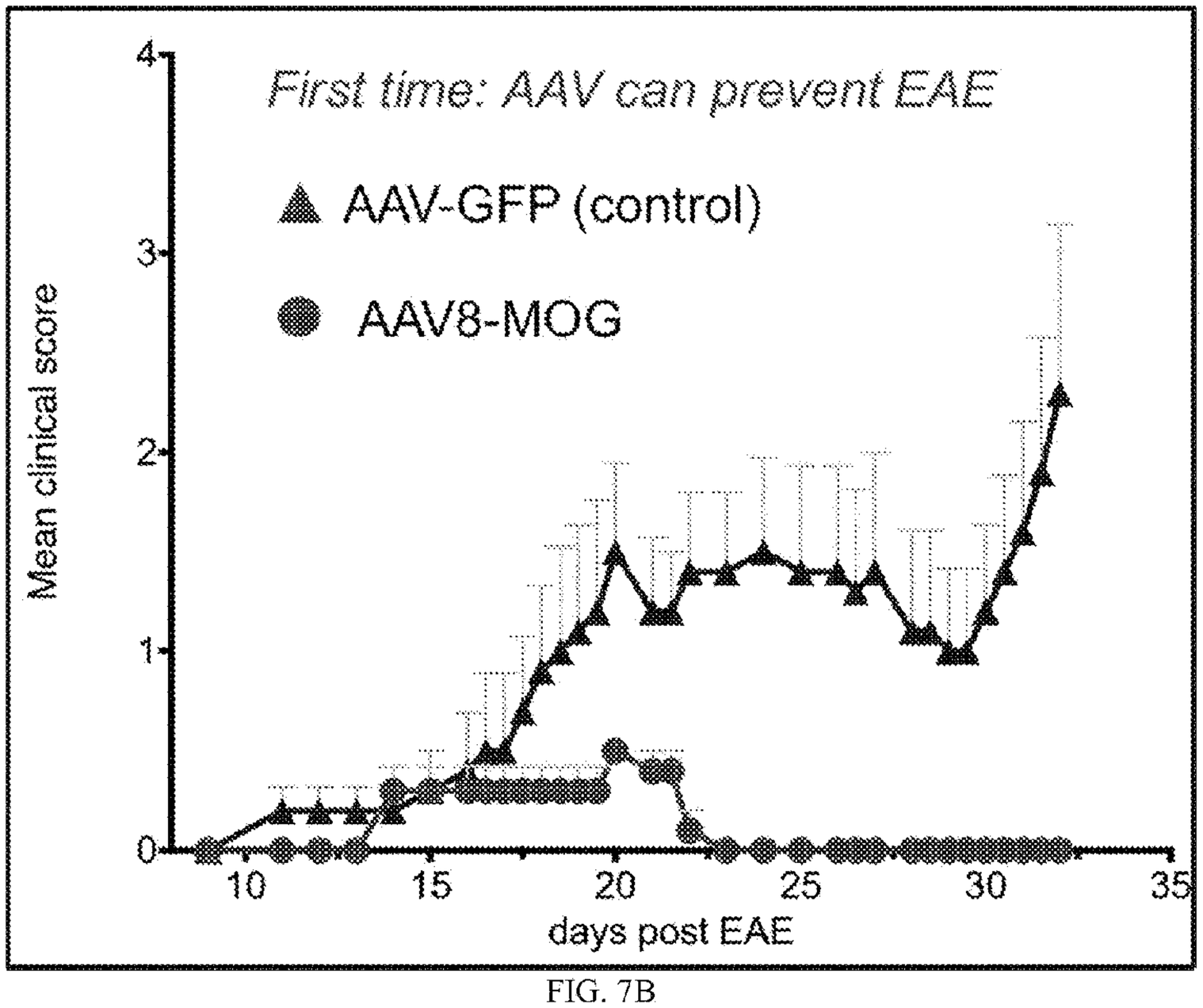

First time: AAV can prevent EAE
AAV-GFP (control)
AAV8-MOG
Mean clinical score
0
1
2
3
4
10
15
20
25
30
35
days post EAE

FIG. 7B

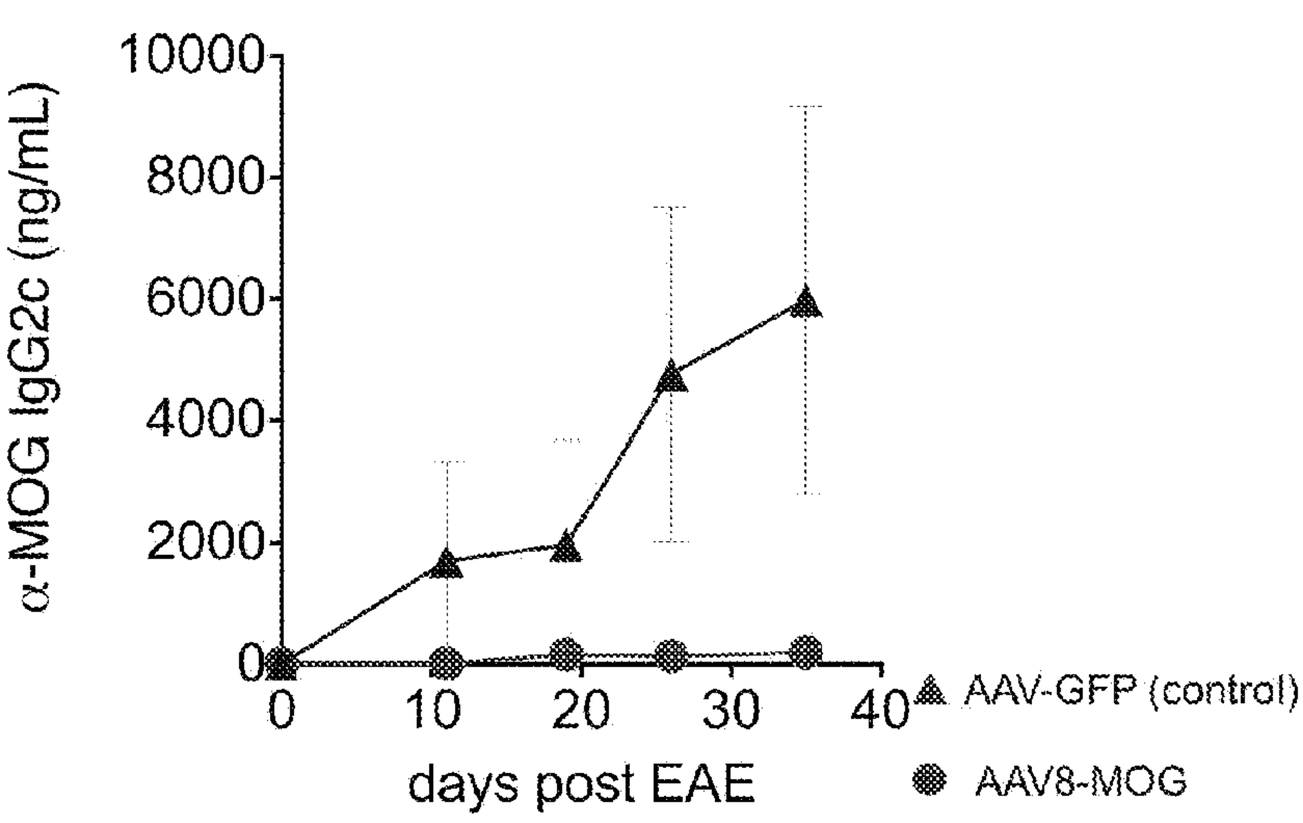

α-MOG IgG2c (ng/mL)
0
2000
4000
6000
8000
10000
0
10
20
30
40
days post EAE
AAV-GFP (control)
AAV8-MOG

FIG. 7C

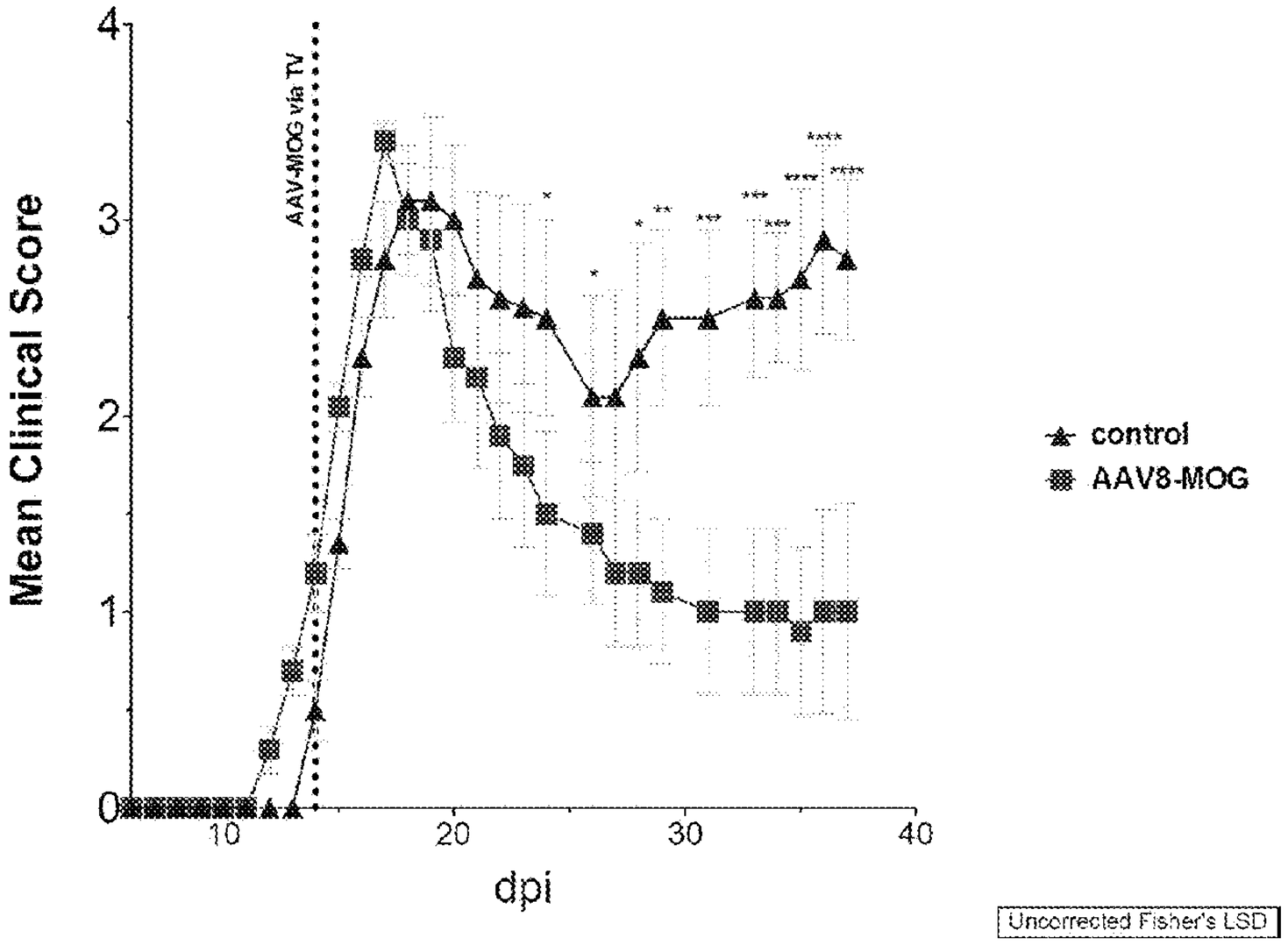

Mean Clinical Score
4
3
2
1
0
AAV-MOG via TV
10
20
30
40
dpi
control
AAV8-MOG
Uncorrected Fisher's LSD

FIG. 8

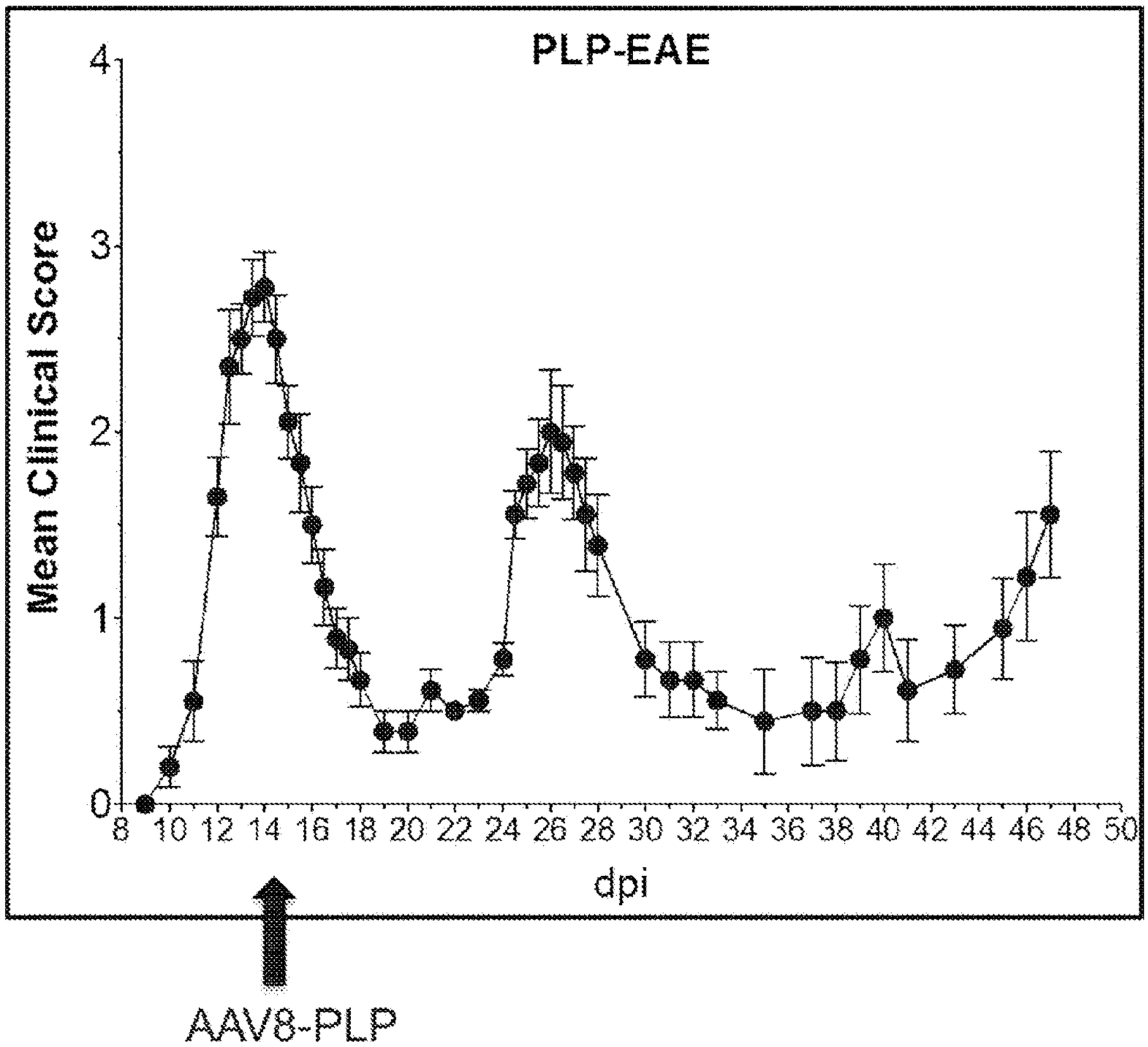

PLP-EAE
Mean Clinical Score
0
1
2
3
4
8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
dpi
AAV8-PLP

FIG. 9

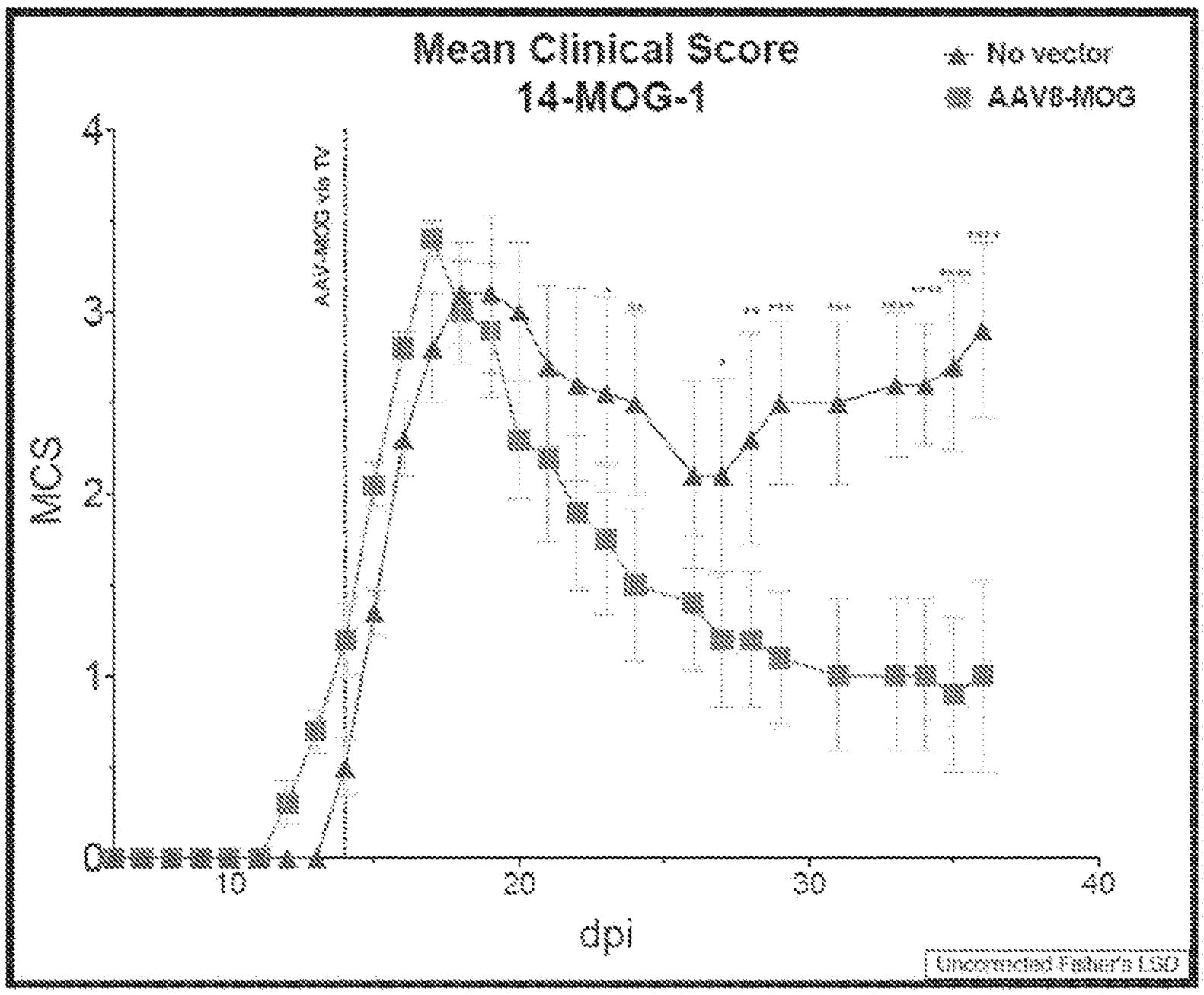

Mean Clinical Score
14-MOG-1
No vector
AAV8-MOG
AAV-MOG via IV
MCS
4
3
2
1
0
10
20
30
40
dpi
Uncorrected Fisher's LSD

FIG. 10

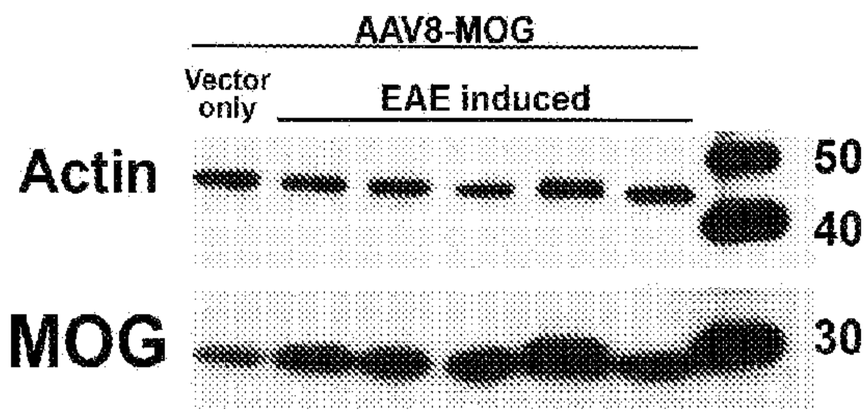
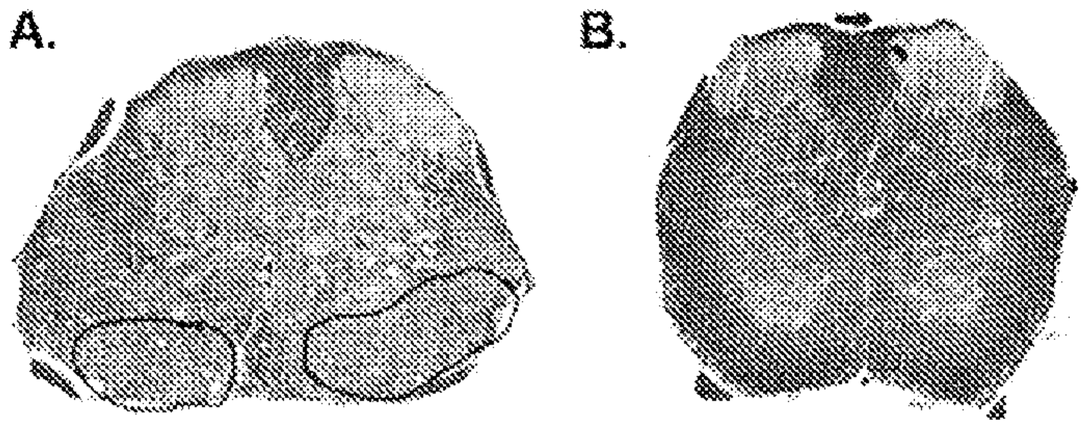
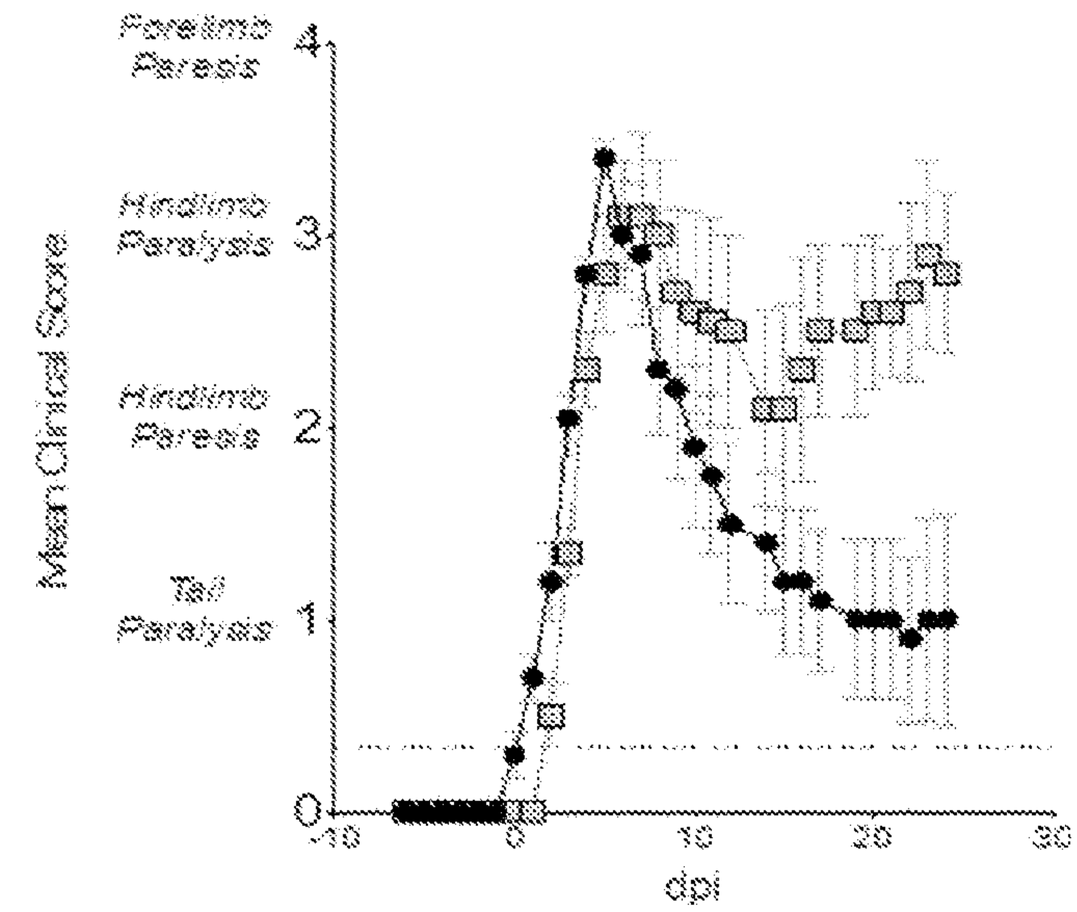

AAV8-MOG
Vector only
EAE induced
Actin
MOG
50
40
30

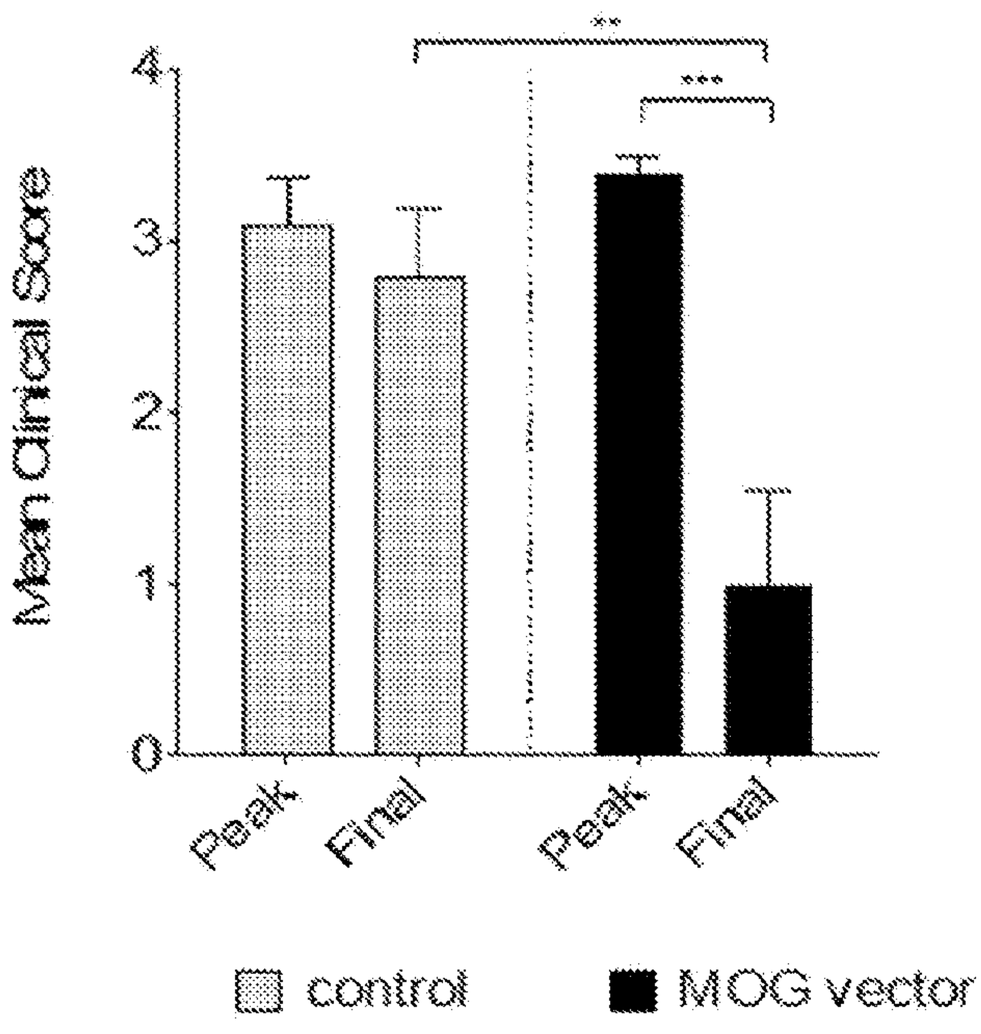
FIG. 11



EAE induced	+	-
Control Vector	+	+
MCS	3.25	0

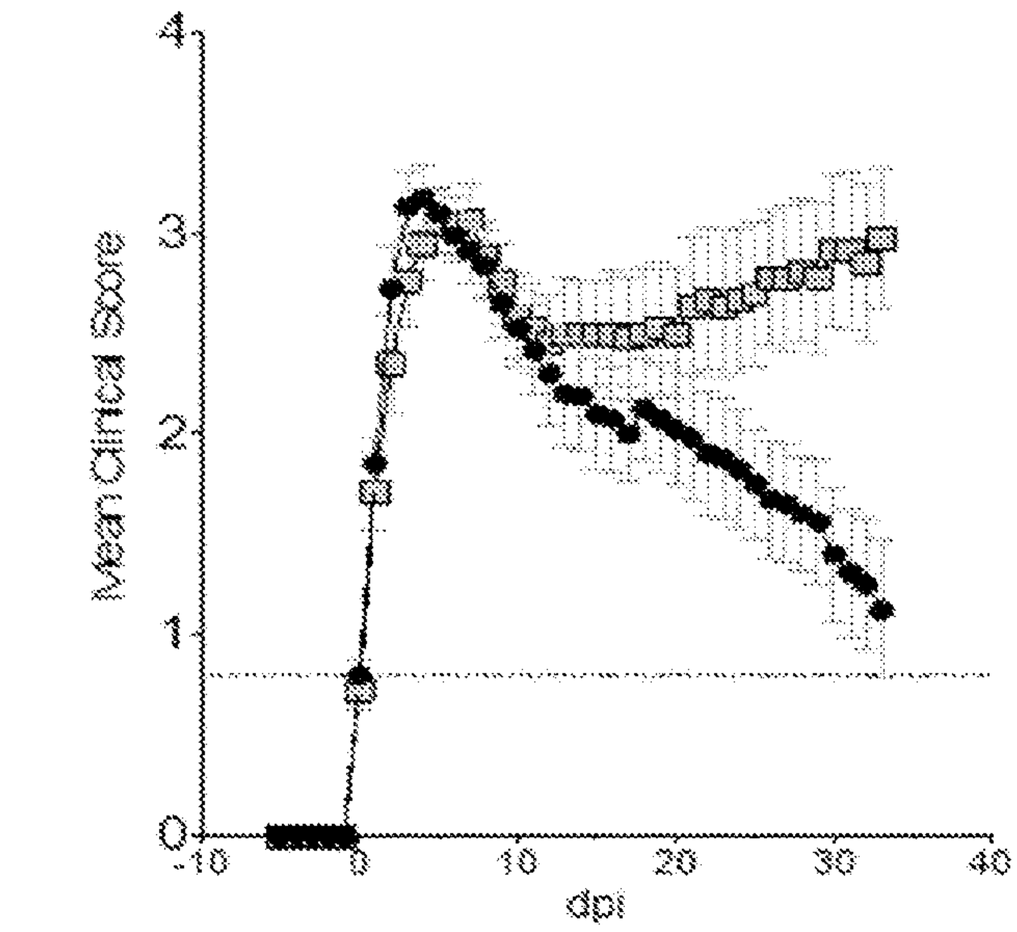
FIG. 12

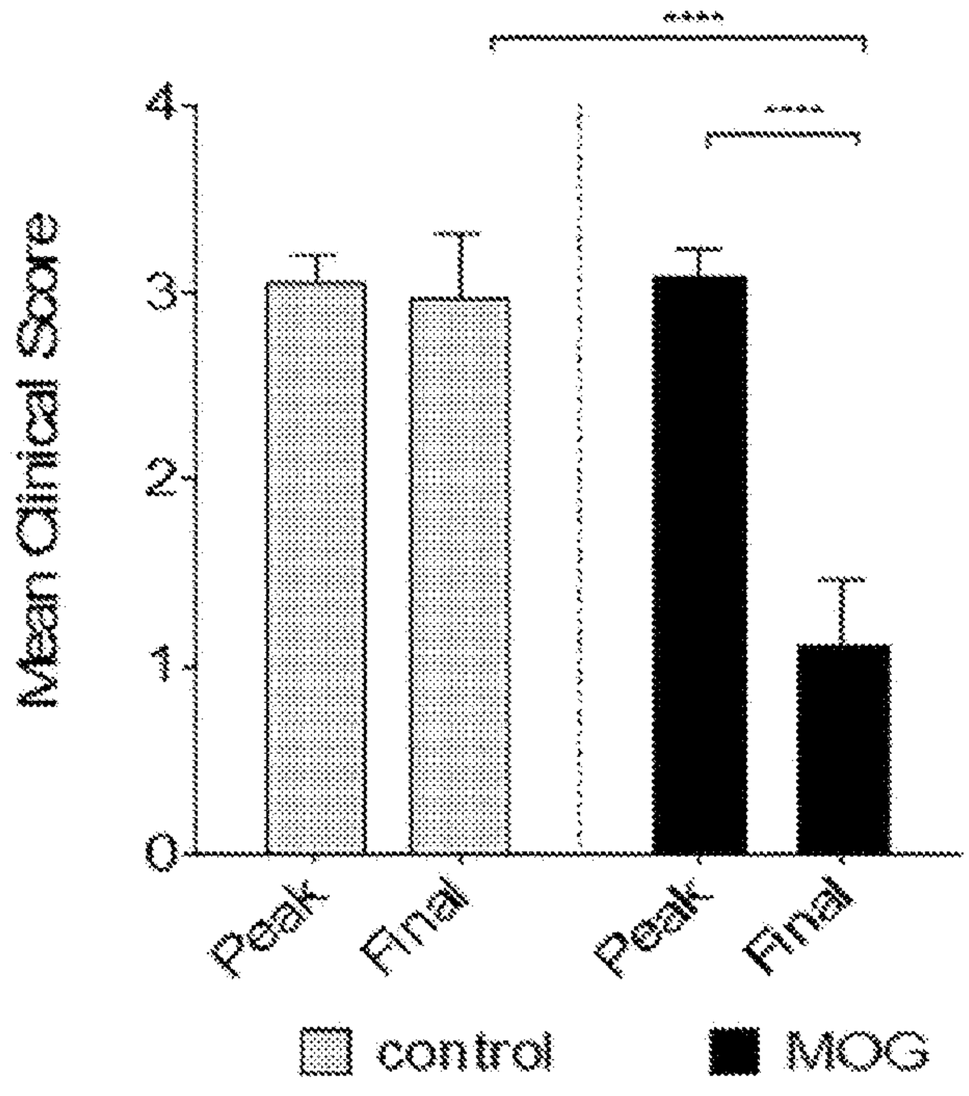




0.3 Mean Clinical Score at time of injection

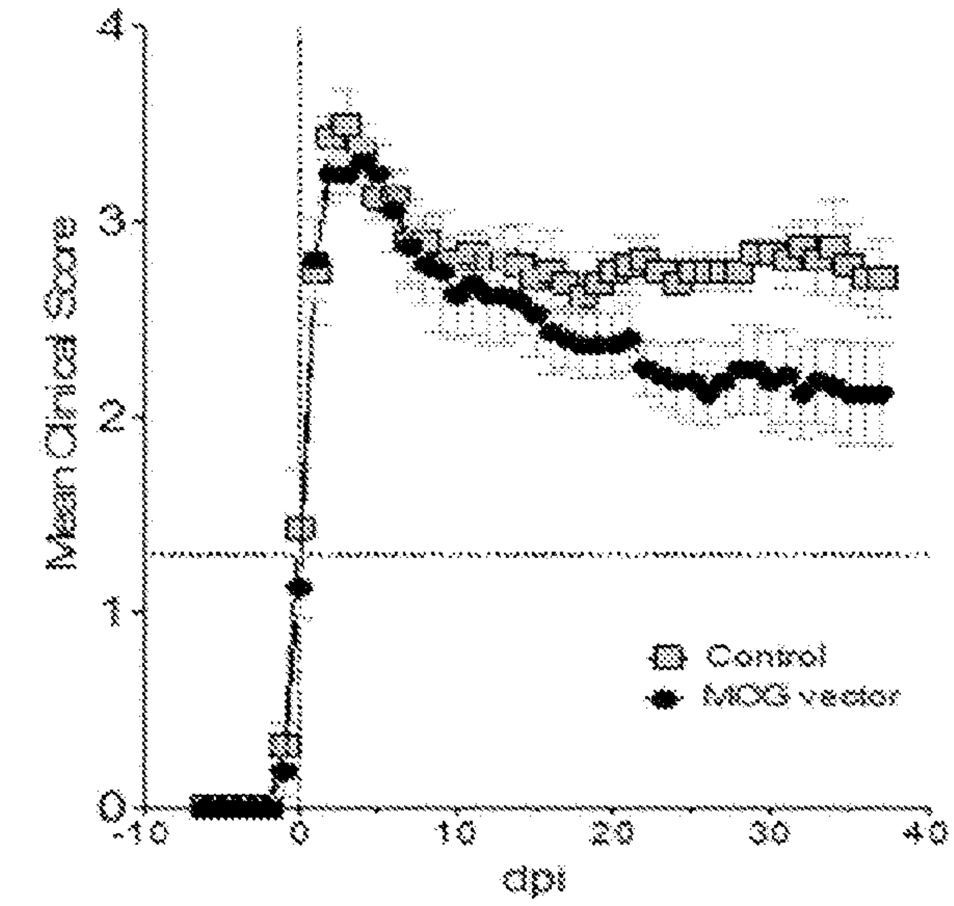
FIG. 13A

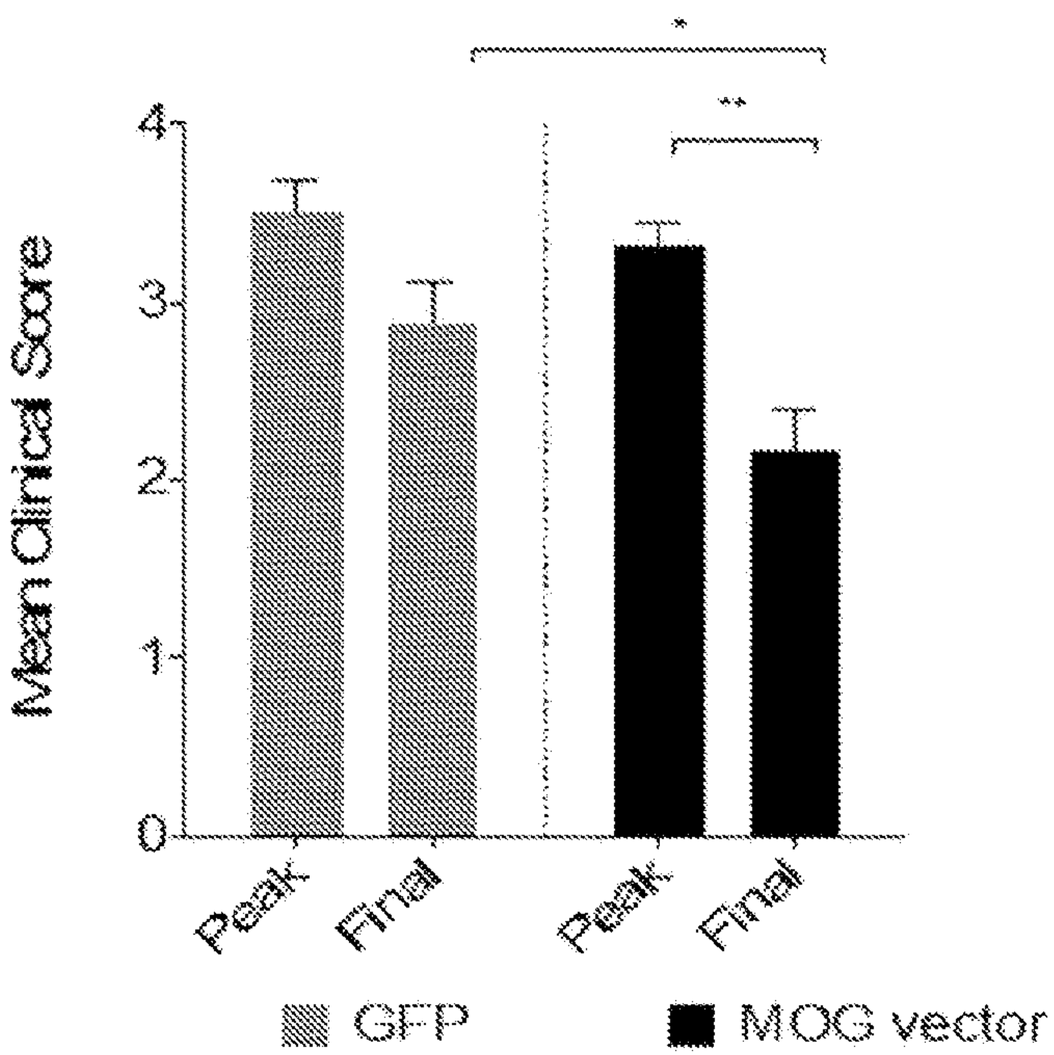




0.8 Mean Clinical Score at time of injection

FIG. 13B





1.3 Mean Clinical Score at time of injection

FIG. 13C

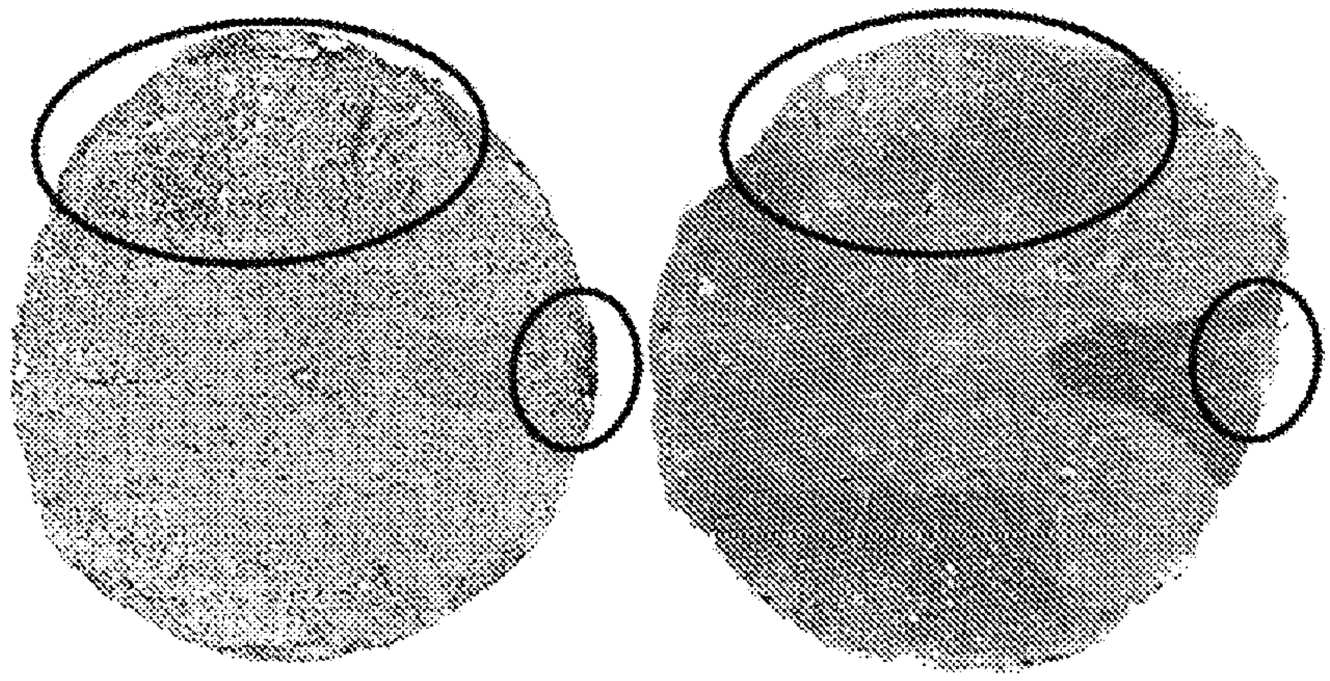


FIG. 14A

FIG. 14B

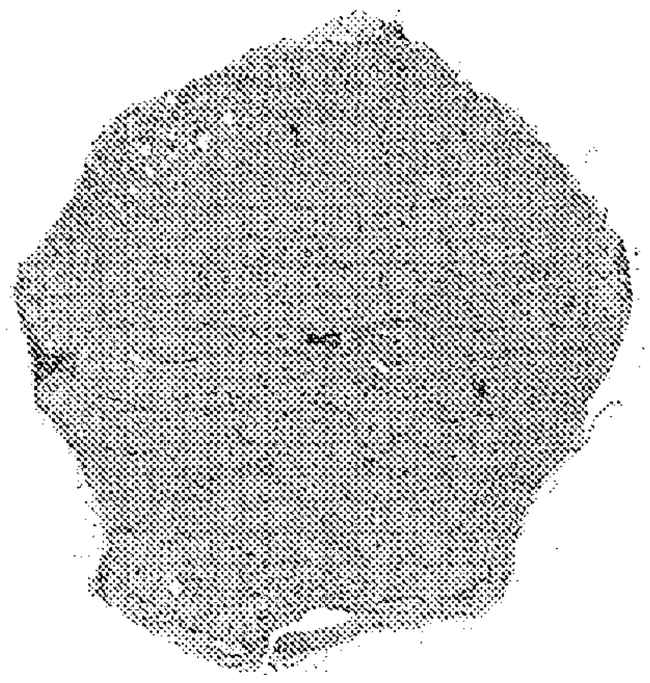


FIG. 15A

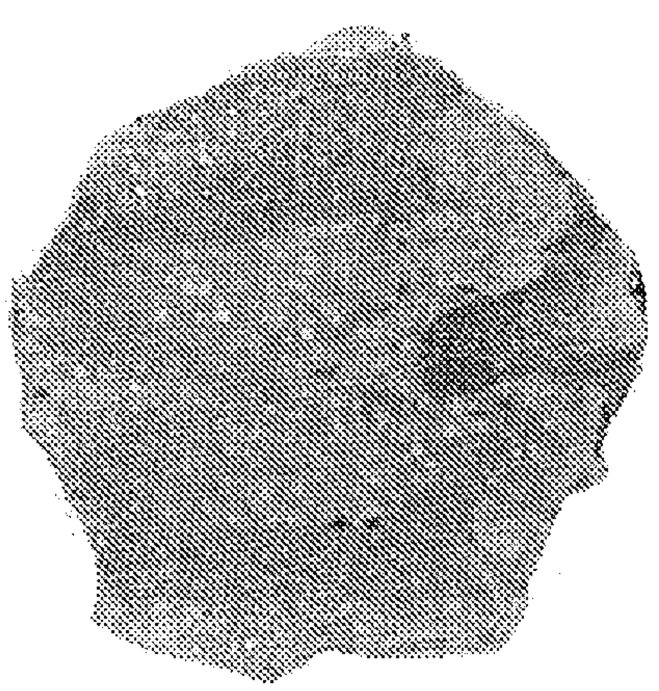


FIG. 15B

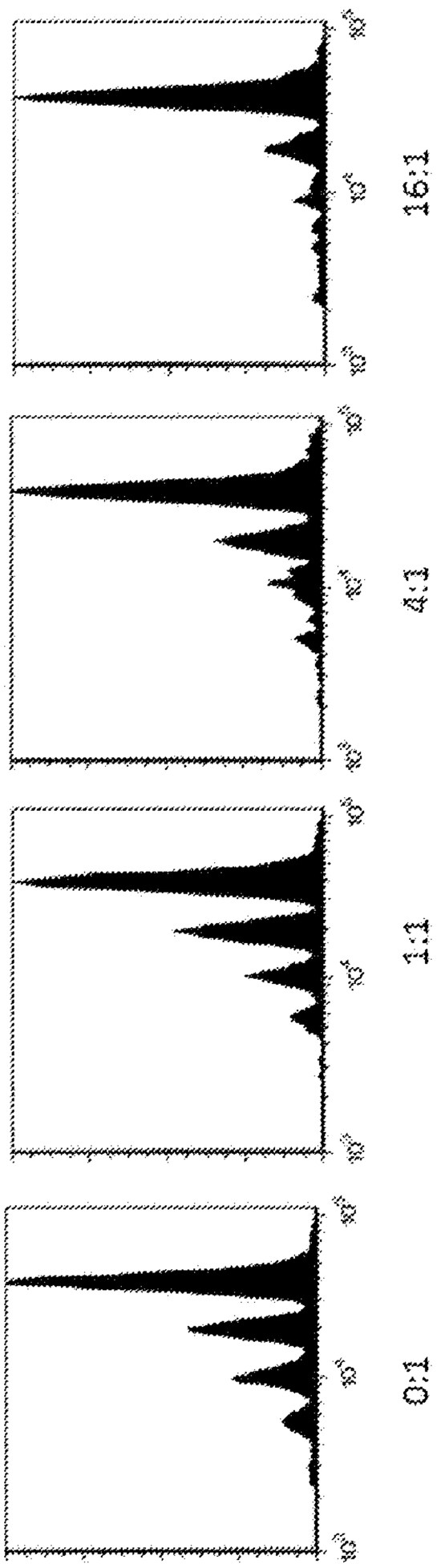


FIG. 16A

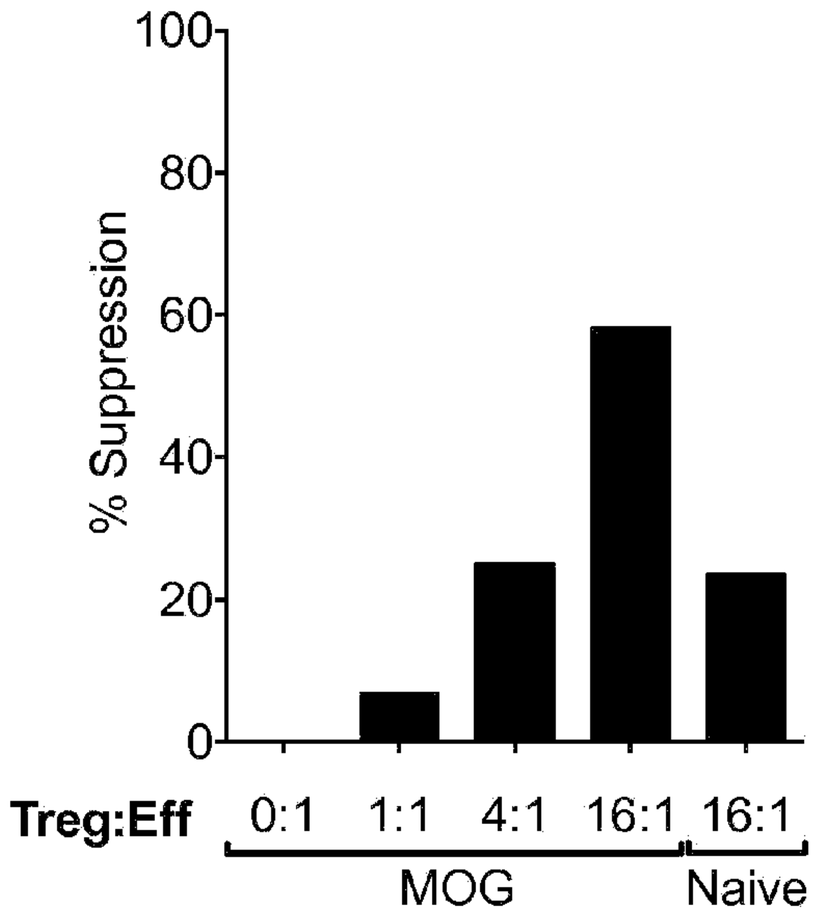

100
80
60
40
20
0
% Suppression
Treg:Eff
0:1
1:1
4:1
16:1
16:1
MOG
Naive

FIG. 16B

Gated on Live CD4+

FIG. 17A

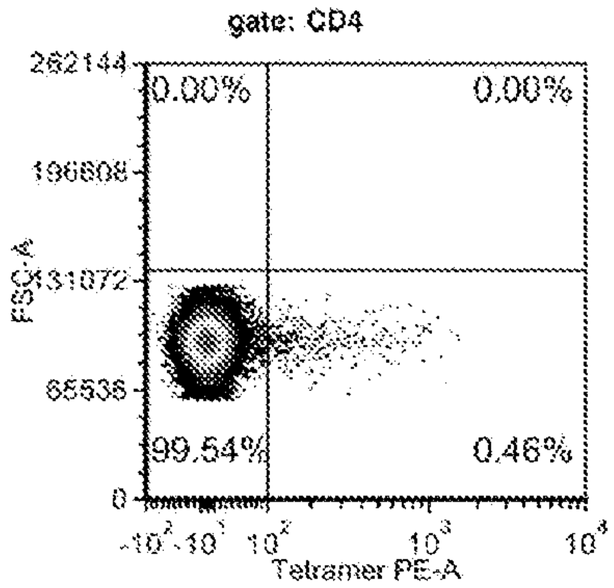
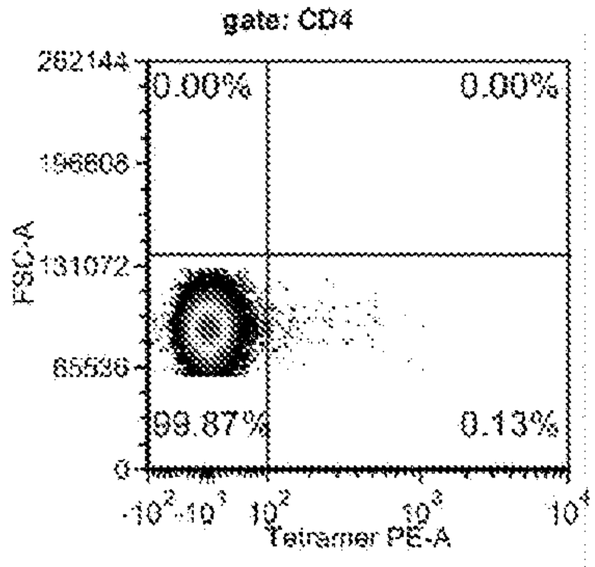


FIG. 17B



Gated on Live CD4+FoxP3+CD25+ (Tregs)

FIG. 17C

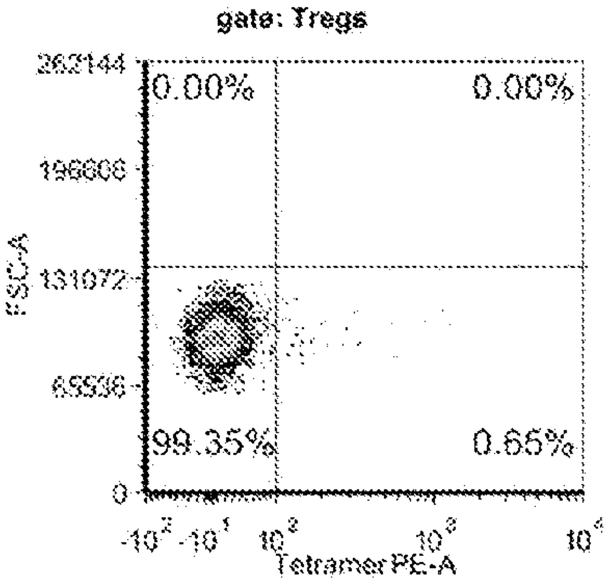
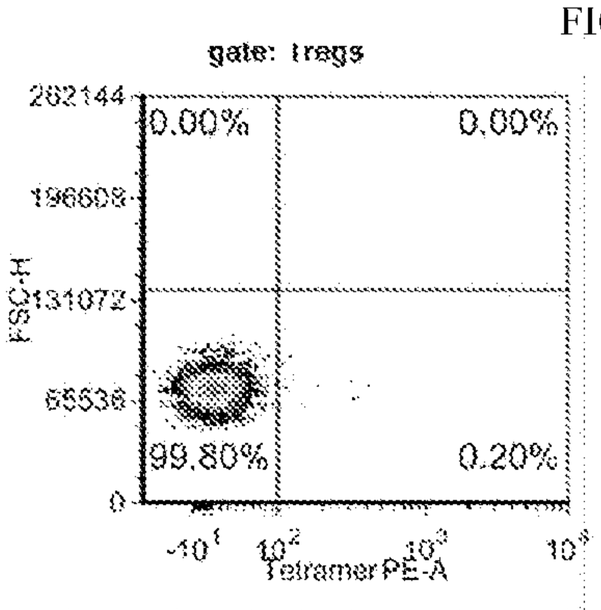
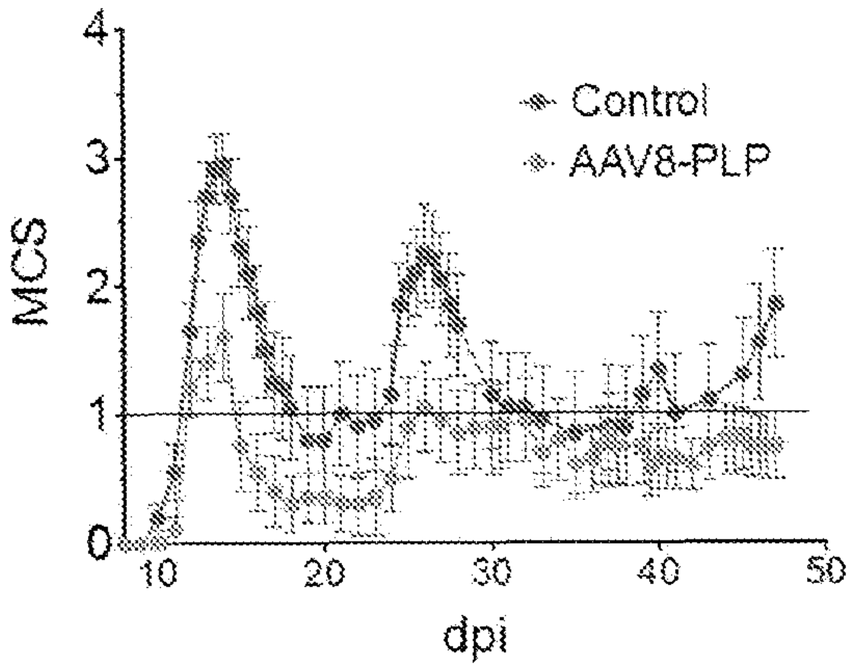


FIG. 17D



MOG Tetramer

Control Tetramer



	Mean Clinical Score	
dpi	Control	AAV8-PLP
14	**3.0** (±0.24)	**1.6** (±0.34)
26	**2.3** (±0.39)	**1.1** (±0.35)

FIG. 18

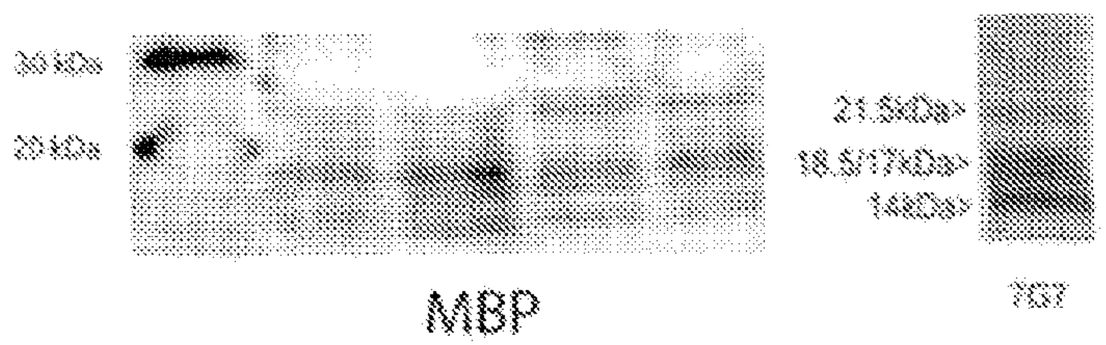


FIG. 19A

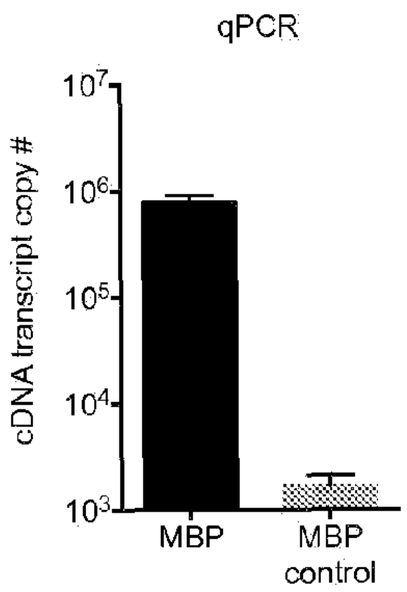


FIG. 19B