"METHODS OF TREATING OR PREVENTING NEUROLOGICAL DISEASES"

RELATED APPLICATION

This application claims priority from US Patent Application No. 61/493,073 entitled "Methods of treating or preventing neurological diseases", filed on 3 June 2011. The entire contents of that application are hereby incorporated by reference.

SEQUENCE LISTING

A sequence listing is filed in electronic form with this application. The entire contents of the sequence listing is hereby incorporated by reference.

FIELD

The present disclosure relates to methods for treating or preventing neurological diseases.

BACKGROUND

Inflammatory neurological diseases are a class of conditions in which a subject's immune system targets or attacks components of the neurological system. These diseases can result from the immune system attacking, for example, neurons, Schwann cells or other cells of the nervous system myelin or neurotransmitters. In some cases, the inflammatory neurological disease may be a complication or a component of an existing disease, e.g., Exemplary inflammatory neurological diseases include multiple sclerosis, systemic lupus erythematosus (SLE), Guillain-Barre syndrome, Lambert-Eaton myasthenic syndrome, myasthenia gravis, transverse myelitis, leukodystrophy or progressive multifocal leukoencephalopathy.

MS is one of the more common inflammatory neurological diseases. It is an inflammatory and demyelinating degenerative disease of the human central nervous system (CNS). It is a worldwide disease that affects approximately 300,000 people in the United States alone. The majority of people affected by MS (about 70%-80% of cases) show onset between 20 and 40 years of age. MS is a heterogeneous disorder based on clinical course, magnetic resonance imaging (MRI) scan assessment, and pathology analysis of biopsy and autopsy material. The disease manifests itself in a large number of possible combinations of deficits, including spinal cord, brainstem, cranial nerve, cerebellar, cerebral, and cognitive syndromes. Progressive disability is

the fate of most patients with MS. About half of MS patients require a cane to walk within 15 years of disease onset.

MS presents in most cases (about 80%) with clinical relapses characterized by fully or partially-reversible focal neurological deficits. This form of MS is known as relapsing-remitting MS (RRMS), and is dominated by inflammation and oedema. Active inflammation of the CNS is visualized as gadolinium enhancing white matter lesions on MRI. After a median of about 39 years, about half of RRMS cases gradually accumulate irreversible neurologic deficits in the absence of clinical relapses or new white matter lesions as detected by MRI. This stage of disease is known as secondary progressive MS (SPMS) or chronic disease. The 20% of patients who do not present with RRMS present with progressive clinical deterioration from the onset of disease, which is known as primary progressive MS (PPMS), which is another form of chronic disease.

Currently, acute MS relapses are usually treated with high-dose, short-term intravenous corticosteroids. This treatment shortens relapse duration but does not improve the degree of recovery or the long-term course of disease. There are currently several approved disease-modifying therapies approved in USA, which are intended to lower the clinical relapse rate, extend the time to next relapse and/or reduce the accumulation of new lesions on MRI. However, these therapies are only moderately effective for treating MS, particularly during the relapsing-remitting phase. These treatments also merely retard the progression of disease and do not result in remyelination.

SLE is an inflammatory disease affecting various organ systems in the body. Subjects suffering from SLE can develop various neurological disorders such as headaches, personality changes, organic brain syndrome, peripheral neuropathies, sensory neuropathy, psychological problems including paranoia, mania, and schizophrenia, seizures, transverse myelitis, and paralysis and stroke. Some of these changes can be brought on by antiphospholipid antibodies (e.g., anti-cardiolipin antibodies), which can bind to cells of the central nervous system and disrupt function and/or thrombosis.

Common pharmalogical treatments for lupus include the use of corticosteroids or immunosuppressive drugs, both of which have undesirable side effects and merely treat the symptoms as they occur.

Other inflammatory neurological diseases are treated using, for example, immunosuppressive drugs, corticosteroids, plasmapheresis or intravenous immunoglobulin, each of which carry a risk of infection or other adverse side effect.

It will therefore be apparent to those skilled in the art that there is a need in the art for new therapies useful for treating inflammatory neurological diseases.

SUMMARY

The inventors have studied the effect of STRO-1$^+$ multipotential cell preparations in an accepted animal models of an inflammatory neurological disease, i.e., chronic paralytic experimental inflammatory encephalomyelitis (EAE). The inventors found that STRO-1+ cells administered after induction of EAE reduced the severity of the disease.

The inventors also found that STRO-1$^+$ cells prevented an immune response against an antigen by T cells derived from an animal previously immunized with the antigen.

The present disclosure provides a method for treating or preventing an inflammatory neurological disease, the method comprising administering to the subject a population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom.

In one example, the inflammatory neurological disease is associated with or caused by a T cell response to an inflammatory stimulus.

In one example, the method comprises administering a population of cells enriched for STRO-1^{bright} cells and/or progeny thereof and/or soluble factors derived therefrom.

In one example, the inflammatory neurological disease is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, Guillain-Barre syndrome, Lambert-Eaton myasthenic syndrome, myasthenia gravis, transverse myelitis, leukodystrophy and progressive multifocal leukoencephalopathy.

In one example, the disease is systemic lupus erythematosus.

In another example, the disease is multiple sclerosis. In one example, the disease is a chronic progressive form of multiple sclerosis. In another example, the disease is a relapsing-remitting form of multiple sclerosis.

In one example, the method comprises administering a population of cells enriched for STRO-1^{bright} cells and/or progeny thereof and/or soluble factors derived therefrom. In one example, the progeny are additionally enriched for STRO-1^{bright} cells.

Exemplary cells and/or progeny additionally express tissue non-specific alkaline phosphatase (TNAP) and/or heat shock protein 90β (HSP90β) and/or CD146.

In one example, the population of cells is derived from bone marrow or dental pulp.

In one example, the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered systemically. For example, the population of cells enriched for Stro-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom may be administered intravenously, intra-arterially, intramuscularly, subcutaneously, into an aorta, into an atrium or ventricle of the heart or into a blood vessel connected to an organ affected by the inflammatory neurological disease. For example, the population and/or progeny and/or soluble factors are administered intravenously.

In another example, the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered into cerebral spinal fluid or into the central nervous system.

In a further example, the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered to a site of disease, e.g., to a site of myelin degeneration.

In the case of a relapsing-remitting disease (e.g., relapsing-remitting MS), the cells can be administered during disease relapse to prevent or delay relapse of the disease.

In one example, the method comprises administering an effective amount of the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom. In one example, the effective amount is an amount sufficient to increase the number of regulatory T (Treg) cells in the subject and/or at the site of pathogenesis.

An exemplary method described herein according to any example, comprises administering a dose of the population and/or the progeny and/or the soluble factors sufficient to improve a clinical measure of the inflammatory neurological disease and/or to reduce or prevent an immune response against an antigen associated with the inflammatory neurological disease.

In one example, the method comprises administering an effective dose or a therapeutically effective dose of the population and/or progeny and/or soluble factors.

In one example, the method comprises administering between 1×10^4 to 5×10^6 STRO-1$^+$ cells and/or progeny thereof per kg. For example, the method comprises administering between 1×10^5 to 1×10^6 STRO-1$^+$ cells and/or progeny thereof per kg. For example, the method comprises administering between 2×10^5 to 8×10^5 STRO-1$^+$ cells and/or progeny thereof per kg. For example, the method comprises administering

about 2 x 10^5 STRO-1^+ cells and/or progeny thereof per kg or about 4 x 10^5 STRO-1^+ cells and/or progeny thereof per kg or about 8 x 10^5 STRO-1^+ cells and/or progeny thereof per kg.

In one example, a method described herein according to any example, comprises administering a low dose of STRO-1^+ cells and/or progeny thereof. For example, the low dose of STRO-1^+ cells and/or progeny thereof comprises between 1 x 10^3 and 3 x 10^5 STRO-1^+ cells and/or progeny thereof per kg.

In one example, the population and/or the progeny and/or the soluble factors are administered a plurality of times. For example, the population and/or the progeny and/or the soluble factors are administered a plurality of times in one week or once every four or more weeks.

In one example, the population and/or the progeny and/or the soluble factors are administered during a remission of an inflammatory neurological condition.

In another example, the population enriched for STRO-1^+ cells and/or progeny thereof are genetically-engineered to express a molecule to block stimulation of T cells and/or the soluble factors are from such genetically-modified cells.

In another example, the population enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors therefrom are administered with a compound to block stimulation of T cells.

The population enriched for STRO-1^+ cells and/or progeny cells can be autogeneic or allogeneic and/or the soluble factors can be derived from autogeneic or allogeneic cells. In one example, the population of cells and/or progeny cells are allogeneic and/or the soluble factors are derived from autogeneic cells.

In one example, the population enriched for STRO-1^+ cells and/or progeny cells have been culture expanded prior to administration and/or prior to obtaining the soluble factors.

In another example, a method described herein further comprises administering an immunosuppressive agent. The immunosuppressive agent may be administered for a time sufficient to permit said transplanted cells to be functional.

The present disclosure also provides a method for preventing an immune response in response to an antigen, the method comprising administering to the subject a population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom.

In one example, the immune response is a T cell-mediated immune response. An exemplary T cell-mediated immune response comprises T cell proliferation.

In one example, the T cell-mediated immune response is suppressed in response to a specific antigen and a T cell-mediated immune response in response to another antigen is not suppressed.

In one example, the subject has previously raised an immune response to the antigen and the population, progeny and/or soluble factors suppress a further immune response to the antigen.

In one example, the population, progeny and/or soluble factors are administered after the subject raises an immune response to the antigen to thereby prevent a further immune response to the antigen.

In one example, the immune response is suppressed for at least about 24 days following administration of the population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom.

The present disclosure also provides a method for inducing tolerance to an antigen in a subject, the method comprising administering to the subject a population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom.

In one example of a method described herein the antigen or the specific antigen is one against which an inflammatory response is raised. For example, the inflammatory response is causative of an inflammatory neurological disease.

In one example of a method described herein according to any example, the population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom is administered with a compound that treats or prevents an inflammatory neurological disease. An exemplary compound is glatiramer acetate and/or beta interferon.

The compound can be mixed with the population and/or progeny and/or soluble factors or administered at the same time and/or administered before or after the population and/or progeny and/or soluble factors (e.g., such that the compound and the population and/or progeny and/or soluble factors are providing a benefit at the same time).

The present disclosure also provides a population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom for use in the treatment or prevention of an inflammatory neurological disease and/or for suppressing a T cell-mediated immune response against an antigen and/or for inducing tolerance to an antigen.

The present disclosure also provides for use of a population of cells enriched for STRO-1^+ cells and/or progeny thereof and/or soluble factors derived therefrom in the

manufacture of a medicament for treating or preventing an inflammatory neurological disease and/or for suppressing a T cell-mediated immune response against an antigen and/or for inducing tolerance to an antigen.

Each example of the disclosure shall be taken to apply *mutatis mutandis* to a method for reducing, delaying or preventing myelin destruction and/or an inflammatory response against myelin or a component thereof.

Each example of the disclosure shall be taken to apply *mutatis mutandis* to inflammation in the nervous system or a component thereof,

Each example of the disclosure shall be taken to apply *mutatis mutandis* to a method for inducing or promoting remyelination or neurite outgrowth.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Co-expression of TNAP (STRO-3) and the Mesenchymal Precursor Cell Marker, STRO-1^{bright} by Adult Human bone marrow morphonuclear cells (BMMNC). Dual-color immunofluorescence and flow cytometry was performed by incubation of STRO-1 MACS-selected BMMNC and indirectly labeled with a goat anti-murine IgM antibody coupled to FITC (x axis), and STRO-3 mAb (murine IgG1) indirectly labeled with a goat anti-murine IgG coupled to PE (y axis). The dot plot histogram represents 5 x 104 events collected as listmode data. The vertical and horizontal lines were set to the reactivity levels of <1.0% mean fluorescence obtained with the isotype-matched control antibodies, 1B5 (IgG) and 1A6.12 (IgM) treated under the same conditions. The results demonstrate that a minor population of STRO-1^{bright} cells co-expressed TNAP (upper right quadrant) while the remaining STRO-1^{+} cells failed to react with the STRO-3 mAb.

Figure 2. Gene expression profile of STRO-1^{bri} or STRO-1^{dim} progeny of cultured and expanded STRO-1^{bri} MPC. Single cell suspensions of *ex vivo* expanded bone marrow MPC were prepared by trypsin/EDTA treatment. Cells were stained with the STRO-1 antibody which was subsequently revealed by incubation with goat-anti murine IgM-fluorescein isothiocyanate. Total cellular RNA was prepared from purified populations of STRO-1^{dim} or STRO-1^{bri} expressing cells, following fluorescence activated cell sorting (A). Using RNAzolB extraction method, and standard procedures, total RNA was isolated from each subpopulation and used as a template for cDNA synthesis. The expression of various transcripts was assessed by PCR amplification, using a standard protocol as described previously (Gronthos *et al. J Cell Sci. 116*:1827-1835, 2003). Primers sets used in this study are shown in Table 2. Following amplification, each reaction mixture was analyzed by 1.5% agarose gel

electrophoresis, and visualized by ethidium bromide staining (B). Relative gene expression for each cell marker was assessed with reference to the expression of the house-keeping gene, GAPDH, using ImageQant software (C).

Figure 3. STRO-1^{bri} progeny of cultured and expanded STRO-1^{+} MPC express high levels of SDF-1, STRO-1^{dim} progeny do not. (A) MACS-isolated preparations of STRO-1^{+} BMMNCs were partitioned into different STRO-1 subsets according to the regions, STRO-1^{bright} and STRO-$1^{dim/dull}$ using FACS. Total RNA was prepared from each STRO-1 subpopulation and used to construct a STRO-1^{bright} subtraction hybridization library (B-C). Replicate nitrocellulose filters, which have been blotted with representative PCR products amplified from bacterial clones transformed with STRO-1^{bright} subtracted cDNA. The filters were then probed with either [^{32}P] deoxycytidine triphosphate (dCTP)–labeled STRO-1^{bright} (B) or STRO-$1^{dim/dull}$ (C) subtracted cDNA. The arrows indicate differential expression of 1 clone containing a cDNA fragment corresponding to human SDF-1. (D) Reverse transcriptase (RT)–PCR analysis demonstrating the relative expression of SDF-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts in total RNA prepared from freshly MACS/FACS-isolated BMMNC STRO-1 populations prior to culture. bp indicates base pair.

Figure 4 is a graphical representation showing the effect of MPC treatment on mean clinical disease scores in a model of chronic progressive EAE. C57BL/6 Mice were immunised with MOG35-55 on day 0 and then treated with intravenous injections of MPCs on days 8, 10 and 12 after disease induction. The dosage of MPCs is indicated.

Figure 5 is a graphical representation showing that MPC treatment induces dose-dependent reduction in cumulative disease index in chronic progressive EAE (total area under the curve analysis of mean clinical disease score)

Figure 6 is a graphical representation showing the fold change in proliferation of splenocytes isolated from mice immunized with MOG_{35-55} compared to unstimulated splenocytes following stimulation with MOG_{35-55}.

Figure 7 is a graphical representation showing the fold change in proliferation of splenocytes isolated from mice immunized with MOG_{35-55} compared to unstimulated splenocytes following non-specific restimulation with PMA/ionomycin.

KEY TO SEQUENCE LISTING

SEQ ID NO: 1 oligonucleotide for amplifying nucleic acid encoding GAPDH

SEQ ID NO: 2 oligonucleotide for amplifying nucleic acid encoding GAPDH

SEQ ID NO: 3 oligonucleotide for amplifying nucleic acid encoding SDF-1
SEQ ID NO: 4 oligonucleotide for amplifying nucleic acid encoding SDF-1
SEQ ID NO: 5 oligonucleotide for amplifying nucleic acid encoding IL-1β
SEQ ID NO: 6 oligonucleotide for amplifying nucleic acid encoding IL-1β
SEQ ID NO: 7 oligonucleotide for amplifying nucleic acid encoding FLT-1
SEQ ID NO: 8 oligonucleotide for amplifying nucleic acid encoding FLT-1
SEQ ID NO: 9 oligonucleotide for amplifying nucleic acid encoding TNF-α
SEQ ID NO: 10 oligonucleotide for amplifying nucleic acid encoding TNF-α
SEQ ID NO: 11 oligonucleotide for amplifying nucleic acid encoding KDR
SEQ ID NO: 12 oligonucleotide for amplifying nucleic acid encoding KDR
SEQ ID NO: 13 oligonucleotide for amplifying nucleic acid encoding RANKL
SEQ ID NO: 14 oligonucleotide for amplifying nucleic acid encoding RANKL
SEQ ID NO: 15 oligonucleotide for amplifying nucleic acid encoding Leptin
SEQ ID NO: 16 oligonucleotide for amplifying nucleic acid encoding Leptin
SEQ ID NO: 17 oligonucleotide for amplifying nucleic acid encoding CBFA-1
SEQ ID NO: 18 oligonucleotide for amplifying nucleic acid encoding CBFA-1
SEQ ID NO: 19 oligonucleotide for amplifying nucleic acid encoding PPARγ2
SEQ ID NO: 20 oligonucleotide for amplifying nucleic acid encoding PPARγ2
SEQ ID NO: 21 oligonucleotide for amplifying nucleic acid encoding OCN
SEQ ID NO: 22 oligonucleotide for amplifying nucleic acid encoding OCN
SEQ ID NO: 23 oligonucleotide for amplifying nucleic acid encoding MyoD
SEQ ID NO: 24 oligonucleotide for amplifying nucleic acid encoding MyoD
SEQ ID NO: 25 oligonucleotide for amplifying nucleic acid encoding SMMHC
SEQ ID NO: 26 oligonucleotide for amplifying nucleic acid encoding SMMHC
SEQ ID NO: 27 oligonucleotide for amplifying nucleic acid encoding GFAP
SEQ ID NO: 28 oligonucleotide for amplifying nucleic acid encoding GFAP
SEQ ID NO: 29 oligonucleotide for amplifying nucleic acid encoding Nestin
SEQ ID NO: 30 oligonucleotide for amplifying nucleic acid encoding Nestin
SEQ ID NO: 31 oligonucleotide for amplifying nucleic acid encoding SOX9
SEQ ID NO: 32 oligonucleotide for amplifying nucleic acid encoding SOX9
SEQ ID NO: 33 oligonucleotide for amplifying nucleic acid encoding Collagen type X
SEQ ID NO: 34 oligonucleotide for amplifying nucleic acid encoding Collagen type X
SEQ ID NO: 35 oligonucleotide for amplifying nucleic acid encoding Aggrecan
SEQ ID NO: 36 oligonucleotide for amplifying nucleic acid encoding Aggrecan

DETAILED DESCRIPTION

General Techniques and Selected Definitions

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

Each example described herein is to be applied *mutatis mutandis* to each and every other example of the disclosure unless specifically stated otherwise.

Those skilled in the art will appreciate that the disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the disclosure.

The present disclosure is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, peptide synthesis in solution, solid phase peptide synthesis, and immunology. Such procedures are described, for example, in Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Second Edition (1989), whole of Vols I, II, and III; DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL Press, Oxford, whole of text; Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed, 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, ppl-22; Atkinson *et al*, pp35-81; Sproat *et al*, pp 83-115; and Wu *et al*, pp 135-151; 4. Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text; Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text; Perbal, B., A Practical Guide to Molecular Cloning (1984); Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series; J.F. Ramalho Ortigao, "The Chemistry of Peptide Synthesis" In: Knowledge database of Access to Virtual Laboratory website (Interactiva, Germany); Sakakibara, D., Teichman, J., Lien,

E. Land Fenichel, R.L. (1976). *Biochem. Biophys. Res. Commun. 73* 336-342; Merrifield, R.B. (1963). *J. Am. Chem. Soc. 85*, 2149-2154; Barany, G. and Merrifield, R.B. (1979) in The Peptides (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York. 12. Wünsch, E., ed. (1974) Synthese von Peptiden in Houben-Weyls Metoden der Organischen Chemie (Müler, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart; Bodanszky, M. (1984) Principles of Peptide Synthesis, Springer-Verlag, Heidelberg; Bodanszky, M. & Bodanszky, A. (1984) The Practice of Peptide Synthesis, Springer-Verlag, Heidelberg; Bodanszky, M. (1985) *Int. J. Peptide Protein Res. 25*, 449-474; Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications); and Animal Cell Culture: Practical Approach, Third Edition (John R. W. Masters, ed., 2000), ISBN 0199637970, whole of text.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source. In the context of soluble factors derived from STRO-1$^+$ cells and/or progeny cells thereof, this term shall be taken to mean one or more factors, e.g., proteins, peptides, carbohydrates, etc, produced during *in vitro* culturing of STRO-1$^+$ cells and/or progeny cells thereof.

As used herein, the term "inflammatory neurological disease" shall be taken to include any disorder characterized by a defect in neuronal signaling and/or neuronal dysfunction and/or neuronal cell death resulting from an inflammatory response, and, in some examples, an autoimmune response. In one example, an inflammatory neurological disorder is a disorder associated with or caused by myelin degeneration and/or autoantibodies against a component of the nervous system, such as, for example a component of myelin or a phospholipid or a ganglioside. An inflammatory neurological disease may be a primary disease or may be a complication of an existing disease, e.g., in some cases of SLE.

As used herein, the term "effective amount" shall be taken to mean a sufficient quantity of STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom to reduce an inflammatory response in a subject that causes or is associated with a neurological disease. For example, an effective amount of STRO-1$^+$ cells and/or

progeny cells thereof and/or soluble factors derived therefrom may reduce lesions in the brain or spinal cord, e.g., as detectable using magnetic resonance imaging (MRI) and/or autoantibodies against myelin and/or oligoclonal bands in CSF.

As used herein, the term "therapeutically effective amount" shall be taken to mean a sufficient quantity of STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom to reduce or inhibit one or more symptoms of a clinical inflammatory neurological disease.

As used herein, the term "prophylactically effective amount" shall be taken to mean a sufficient quantity of STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom to prevent or inhibit or delay the onset of one or more detectable symptoms of a clinical inflammatory neurological disease.

As used herein, the term "low dose" shall be understood to mean an amount of STRO-1$^+$ cells and/or progeny thereof less than $0.7x10^6$, yet still sufficient to lipid and/or lipoprotein levels in a subject that cause(s) or is(are) associated with an inflammatory neurological disease and/or to treat or prevent a an inflammatory neurological disease. For example, a low dose comprises 0.5×10^6 or fewer cells, or 0.4×10^6 or fewer cells or 0.3×10^6 or fewer cells or 0.2×10^6 or fewer cells.

As used herein, the term "treat" or "treatment" or "treating" shall be understood to mean administering a therapeutically effective amount of soluble factors and/or cells and reducing or inhibiting at least one symptom of a clinical condition associated with or caused by an inflammatory neurological condition.

As used herein, the term "prevent" or "preventing" or "prevention" shall be taken to mean administering a prophylactically effective amount of soluble factors and/or cells and stopping or hindering or delaying the development or progression of at least one symptom of a clinical inflammatory neurological condition.

As used herein, the term "soluble factors" shall be taken to mean any molecule, e.g., protein, peptide, glycoprotein, glycopeptide, lipoprotein, lipopeptide, carbohydrate, etc. produced by STRO-1$^+$ cells and/or progeny thereof that are water soluble. Such soluble factors may be intracellular and/or secreted by a cell. Such soluble factors may be a complex mixture (e.g., supernatant) and/or a fraction thereof and/or may be a purified factor. In one example of the present disclosure soluble factors are or are contained within supernatant. Accordingly, any example herein directed to administration of one or more soluble factors shall be taken to apply *mutatis mutandis* to the administration of supernatant.

As used herein, the term "supernatant" refers to the non-cellular material produced following the *in vitro* culturing of mesenchymal precursor cells, and/or

progeny cells thereof, in a suitable medium, such as liquid medium. Typically, the supernatant is produced by culturing the cells in the medium under suitable conditions and time, followed by removing the cellular material by a process such as centrifugation. The supernatant may or may not have been subjected to further purification steps before administration. In one example, the supernatant comprises less than 10^5, such as less than 10^4, for example, less than 10^3 and such as no live cells.

As used herein, the term "prevents an immune response to an antigen" will be understood to mean that a population and/or progeny and/or soluble factors described herein according to any example delays and/or reduces and/or stops development of an immune response, as opposed to suppressing a pre-existing an immune response. In some examples herein, an example of the disclosure directed to preventing an immune response to an antigen shall be taken to apply *mutatis mutandis* to reducing or inhibiting an existing immune response to an antigen.

As used herein, the term "normal or healthy individual" shall be taken to mean a subject that does not suffer from an inflammatory neurological condition as assessed by any method known in the art and/or described herein.

As used herein, the term "glatiramer acetate" will be understood to mean an immunomodulator drug comprising a random polymer of four amino acids found in myelin basic protein, namely glutamic acid, lysine, alanine, and tyrosine currently sold under the trade name Copaxone.

STRO-1$^+$ Cells or Progeny Cells, and Supernatant or One or More Soluble Factors Derived Therefrom

STRO-1$^+$ cells are cells found in bone marrow, blood, dental pulp cells, adipose tissue, skin, spleen, pancreas, brain, kidney, liver, heart, retina, brain, hair follicles, intestine, lung, lymph node, thymus, bone, ligament, tendon, skeletal muscle, dermis, and periosteum; and are capable of differentiating into germ lines such as mesoderm and/or endoderm and/or ectoderm.

In one example, the STRO-1$^+$ cells are multipotential cells which are capable of differentiating into a large number of cell types including, but not limited to, adipose, osseous, cartilaginous, elastic, muscular, and fibrous connective tissues. The specific lineage-commitment and differentiation pathway which these cells enter depends upon various influences from mechanical influences and/or endogenous bioactive factors, such as growth factors, cytokines, and/or local microenvironmental conditions established by host tissues. STRO-1$^+$ multipotential cells are thus non-hematopoietic

progenitor cells which divide to yield daughter cells that are either stem cells or are precursor cells which in time will irreversibly differentiate to yield a phenotypic cell.

In one example, the STRO-1$^+$ cells are enriched from a sample obtained from a subject, e.g., a subject to be treated or a related subject or an unrelated subject (whether of the same species or different). The terms "enriched", "enrichment" or variations thereof are used herein to describe a population of cells in which the proportion of one particular cell type or the proportion of a number of particular cell types is increased when compared with an untreated population of the cells (e.g., cells in their native environment). In one example, a population enriched for STRO-1$^+$ cells comprises at least about 0.1% or 0.5% or 1% or 2% or 5% or 10% or 15% or 20% or 25% or 30% or 50% or 75% STRO-1$^+$ cells. In this regard, the term "population of cells enriched for STRO-1$^+$ cells" will be taken to provide explicit support for the term "population of cells comprising X% STRO1$^+$ cells", wherein X% is a percentage as recited herein. The STRO-1$^+$ cells can, in some examples, form clonogenic colonies, e.g. CFU-F (fibroblasts) or a subset thereof (e.g., 50% or 60% or 70% or 70% or 90% or 95%) can have this activity.

In one example, the population of cells is enriched from a cell preparation comprising STRO-1$^+$ cells in a selectable form. In this regard, the term "selectable form" will be understood to mean that the cells express a marker (e.g., a cell surface marker) permitting selection of the STRO-1$^+$ cells. The marker can be STRO-1, but need not be. For example, as described and/or exemplified herein, cells (e.g., MPCs) expressing STRO-2 and/or STRO-3 (TNAP) and/or STRO-4 and/or VCAM-1 and/or CD146 and/or 3G5 also express STRO-1 (and can be STRO-1^{bright}). Accordingly, an indication that cells are STRO-1$^+$ does not mean that the cells are selected by STRO-1 expression. In one example, the cells are selected based on at least STRO-3 expression, e.g., they are STRO-3$^+$ (TNAP$^+$).

Reference to selection of a cell or population thereof does not require selection from a specific tissue source. As described herein STRO-1$^+$ cells can be selected from or isolated from or enriched from a large variety of sources. That said, in some examples, these terms provide support for selection from any tissue comprising STRO-1$^+$ cells (e.g., MPCs) or vascularized tissue or tissue comprising pericytes (e.g., STRO-1$^+$ pericytes) or any one or more of the tissues recited herein.

In one example, the cells used in the present disclosure express one or more markers individually or collectively selected from the group consisting of TNAP$^+$, VCAM-1$^+$, THY-1$^+$, STRO-4$^+$ (HSP-90β), STRO-2$^+$, CD45$^+$, CD146$^+$, 3G5$^+$ or any combination thereof.

By "individually" is meant that the disclosure encompasses the recited markers or groups of markers separately, and that, notwithstanding that individual markers or groups of markers may not be separately listed herein the accompanying claims may define such marker or groups of markers separately and divisibly from each other.

By "collectively" is meant that the disclosure encompasses any number or combination of the recited markers or groups of peptides, and that, notwithstanding that such numbers or combinations of markers or groups of markers may not be specifically listed herein the accompanying claims may define such combinations or sub-combinations separately and divisibly from any other combination of markers or groups of markers.

In one example, the STRO-1^{+} cells are STRO-1^{bright} (*syn.* STRO-1^{bri}). In one example, the Stro-1^{bri} cells are preferentially enriched relative to STRO-1^{dim} or STRO-1^{intermediate} cells.

In one example, the STRO-1^{bright} cells are additionally one or more of TNAP^{+}, VCAM-1^{+}, THY-1^{+}, STRO-4^{+} (HSP-90β), STRO-2^{+} and/or CD146^{+}. For example, the cells are selected for one or more of the foregoing markers and/or shown to express one or more of the foregoing markers. In this regard, a cell shown to express a marker need not be specifically tested, rather previously enriched or isolated cells can be tested and subsequently used, isolated or enriched cells can be reasonably assumed to also express the same marker.

In one example, the mesenchymal precursor cells are perivascular mesenchymal precursor cells as defined in WO 2004/85630. For example, the mesenchymal precursor cells express a marker of a perivascular cell, e.g., the cells are STRO-1^{+} or STRO-1^{bright} and/or 3G5^{+}. In one example, the cells are or were previously or are progeny of cells that were isolated from vascularized tissue or organs or parts thereof.

A cell that is referred to as being "positive" for a given marker it may express either a low (lo or dim) or a high (bright, bri) level of that marker depending on the degree to which the marker is present on the cell surface, where the terms relate to intensity of fluorescence or other marker used in the sorting process of the cells. The distinction of lo (or dim or dull) and bri will be understood in the context of the marker used on a particular cell population being sorted. A cell that is referred to as being "negative" for a given marker is not necessarily completely absent from that cell. This term means that the marker is expressed at a relatively very low level by that cell, and that it generates a very low signal when detectably labeled or is undetectable above background levels, e.g., levels detected suing an isotype control antibody.

The term "bright", when used herein, refers to a marker on a cell surface that generates a relatively high signal when detectably labeled. Whilst not wishing to be limited by theory, it is proposed that "bright" cells express more of the target marker protein (for example the antigen recognized by STRO-1) than other cells in the sample. For instance, STRO-1^{bri} cells produce a greater fluorescent signal, when labeled with a FITC-conjugated STRO-1 antibody as determined by fluorescence activated cell sorting (FACS) analysis, than non-bright cells (STRO-$1^{dull/dim}$). In one example, "bright" cells constitute at least about 0.1% of the most brightly labeled bone marrow mononuclear cells contained in the starting sample. In other examples, "bright" cells constitute at least about 0.1%, at least about 0.5%, at least about 1%, at least about 1.5%, or at least about 2%, of the most brightly labeled bone marrow mononuclear cells contained in the starting sample. In an example, STRO-1^{bright} cells have 2 log magnitude higher expression of STRO-1 surface expression relative to "background", namely cells that are STRO-1$^-$. By comparison, STRO-1^{dim} and/or STRO-$1^{intermediate}$ cells have less than 2 log magnitude higher expression of STRO-1 surface expression, typically about 1 log or less than "background".

As used herein the term "TNAP" is intended to encompass all isoforms of tissue non-specific alkaline phosphatase. For example, the term encompasses the liver isoform (LAP), the bone isoform (BAP) and the kidney isoform (KAP). In an example, the TNAP is BAP. In an example, TNAP as used herein refers to a molecule which can bind the STRO-3 antibody produced by the hybridoma cell line deposited with ATCC on 19 December 2005 under the provisions of the Budapest Treaty under deposit accession number PTA-7282.

Furthermore, in an example, the STRO-1^+ cells are capable of giving rise to clonogenic CFU-F.

In one example, a significant proportion of the STRO-1^+ multipotential cells are capable of differentiation into at least two different germ lines. Non-limiting examples of the lineages to which the multipotential cells may be committed include bone precursor cells; hepatocyte progenitors, which are multipotent for bile duct epithelial cells and hepatocytes; neural restricted cells, which can generate glial cell precursors that progress to oligodendrocytes and astrocytes; neuronal precursors that progress to neurons; precursors for cardiac muscle and cardiomyocytes, glucose-responsive insulin secreting pancreatic beta cell lines. Other lineages include, but are not limited to, odontoblasts, dentin-producing cells and chondrocytes, and precursor cells of the following: retinal pigment epithelial cells, fibroblasts, skin cells such as keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, smooth and skeletal muscle

cells, testicular progenitors, vascular endothelial cells, tendon, ligament, cartilage, adipocyte, fibroblast, marrow stroma, cardiac muscle, smooth muscle, skeletal muscle, pericyte, vascular, epithelial, glial, neuronal, astrocyte and oligodendrocyte cells.

In another example, the STRO-1$^+$ cells are not capable of giving rise, upon culturing, to hematopoietic cells.

In one example, the cells are taken from the subject to be treated, cultured *in vitro* using standard techniques and used to obtain supernatant or soluble factors or expanded cells for administration to the subject as an autologous or allogeneic composition. In an alternative example, cells of one or more of the established human cell lines are used. In another useful example of the disclosure, cells of a non-human animal (or if the patient is not a human, from another species) are used.

The present disclosure also contemplates use of supernatant or soluble factors obtained or derived from STRO-1$^+$ cells and/or progeny cells thereof (the latter also being referred to as expanded cells) which are produced from *in vitro* culture. Expanded cells of the disclosure may a have a wide variety of phenotypes depending on the culture conditions (including the number and/or type of stimulatory factors in the culture medium), the number of passages and the like. In certain examples, the progeny cells are obtained after about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 passages from the parental population. However, the progeny cells may be obtained after any number of passages from the parental population.

The progeny cells may be obtained by culturing in any suitable medium. The term "medium", as used in reference to a cell culture, includes the components of the environment surrounding the cells. Media may be solid, liquid, gaseous or a mixture of phases and materials. Media include liquid growth media as well as liquid media that do not sustain cell growth. Media also include gelatinous media such as agar, agarose, gelatin and collagen matrices. Exemplary gaseous media include the gaseous phase that cells growing on a petri dish or other solid or semisolid support are exposed to. The term "medium" also refers to material that is intended for use in a cell culture, even if it has not yet been contacted with cells. In other words, a nutrient rich liquid prepared for bacterial culture is a medium. A powder mixture that when mixed with water or other liquid becomes suitable for cell culture may be termed a "powdered medium".

In an example, progeny cells useful for the methods of the disclosure are obtained by isolating TNAP$^+$ STRO-1$^+$ cells from bone marrow using magnetic beads labeled with the STRO-3 antibody, and then culture expanding the isolated cells (see

Gronthos *et al. Blood 85*: 929-940, 1995 for an example of suitable culturing conditions).

In one example, such expanded cells (progeny) (for example, at least after 5 passages) can be TNAP$^-$, CC9$^+$, HLA class I$^+$, HLA class II$^-$, CD14$^-$, CD19$^-$, CD3$^-$, CD11a$^-$c$^-$, CD31$^-$, CD86$^-$, CD34$^-$ and/or CD80$^-$. However, it is possible that under different culturing conditions to those described herein that the expression of different markers may vary. Also, whilst cells of these phenotypes may predominate in the expended cell population it does not mean that there is a minor proportion of the cells do not have this phenotype(s) (for example, a small percentage of the expanded cells may be CC9$^-$). In one example, expanded cells still have the capacity to differentiate into different cell types.

In one example, an expended cell population used to obtain supernatant or soluble factors, or cells *per se*, comprises cells wherein at least 25%, such as at least 50%, of the cells are CC9$^+$.

In another example, an expanded cell population used to obtain supernatant or soluble factors, or cells *per se*, comprises cells wherein at least 40%, such as at least 45%, of the cells are STRO-1$^+$.

In a further example, the expanded cells may express one or more markers collectively or individually selected from the group consisting of LFA-3, THY-1, VCAM-1, ICAM-1, PECAM-1, P-selectin, L-selectin, 3G5, CD49a/CD49b/CD29, CD49c/CD29, CD49d/CD29, CD 90, CD29, CD18, CD61, integrin beta 6-19, thrombomodulin, CD10, CD13, SCF, PDGF-R, EGF-R, IGF1-R, NGF-R, FGF-R, Leptin-R (STRO-2 = Leptin-R), RANKL, STRO-1^{bright} and CD146 or any combination of these markers.

In one example, the progeny cells are Multipotential Expanded STRO-1$^+$ Multipotential cells Progeny (MEMPs) as defined and/or described in WO 2006/032092. Methods for preparing enriched populations of STRO-1$^+$ multipotential cells from which progeny may be derived are described in WO 01/04268 and WO 2004/085630. In an *in vitro* context STRO-1$^+$ multipotential cells will rarely be present as an absolutely pure preparation and will generally be present with other cells that are tissue specific committed cells (TSCCs). WO 01/04268 refers to harvesting such cells from bone marrow at purity levels of about 0.1% to 90%. The population comprising MPCs from which progeny are derived may be directly harvested from a tissue source, or alternatively it may be a population that has already been expanded *ex vivo*.

For example, the progeny may be obtained from a harvested, unexpanded, population of substantially purified STRO-1$^+$ multipotential cells, comprising at least

about 0.1, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80 or 95% of total cells of the population in which they are present. This level may be achieved, for example, by selecting for cells that are positive for at least one marker individually or collectively selected from the group consisting of TNAP, STRO-1^{bright}, $3G5^+$, VCAM-1, THY-1, CD146 and STRO-2.

MEMPS can be distinguished from freshly harvested STRO-1^+ multipotential cells in that they are positive for the marker STRO-1^{bri} and negative for the marker Alkaline phosphatase (ALP). In contrast, freshly isolated STRO-1^+ multipotential cells are positive for both STRO-1^{bri} and ALP. In an example of the present disclosure, at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the administered cells have the phenotype STRO-1^{bri}, ALP^-. In one example the MEMPS are positive for one or more of the markers Ki67, CD44 and/or CD49c/CD29, VLA-3, $\alpha 3\beta 1$. In yet a further example the MEMPs do not exhibit TERT activity and/or are negative for the marker CD18.

The STRO-1^+ cell starting population may be derived from any one or more tissue types set out in WO 01/04268 or WO 2004/085630, namely bone marrow, dental pulp cells, adipose tissue and skin, or perhaps more broadly from adipose tissue, teeth, dental pulp, skin, liver, kidney, heart, retina, brain, hair follicles, intestine, lung, spleen, lymph node, thymus, pancreas, bone, ligament, bone marrow, tendon and skeletal muscle.

It will be understood that in performing the present disclosure, separation of cells carrying any given cell surface marker can be effected by a number of different methods, however, some methods rely upon binding a binding agent (e.g., an antibody or antigen binding fragment thereof) to the marker concerned followed by a separation of those that exhibit binding, being either high level binding, or low level binding or no binding. The most convenient binding agents are antibodies or antibody-based molecules, such as monoclonal antibodies or based on monoclonal antibodies because of the specificity of these latter agents. Antibodies can be used for both steps, however other agents might also be used, thus ligands for these markers may also be employed to enrich for cells carrying them, or lacking them.

The antibodies or ligands may be attached to a solid support to allow for a crude separation. In some examples, the separation techniques maximize the retention of viability of the fraction to be collected. Various techniques of different efficacy may be employed to obtain relatively crude separations. The particular technique employed will depend upon efficiency of separation, associated cytotoxicity, ease and speed of performance, and necessity for sophisticated equipment and/or technical skill.

Procedures for separation may include, but are not limited to, magnetic separation, using antibody-coated magnetic beads, affinity chromatography and "panning" with antibody attached to a solid matrix. Techniques providing accurate separation include but are not limited to FACS. Methods for performing FACS will be apparent to the skilled artisan.

Antibodies against each of the markers described herein are commercially available (e.g., monoclonal antibodies against STRO-1 are commercially available from R&D Systems, USA), available from ATCC or other depositary organization and/or can be produced using art recognized techniques.

The method for isolating STRO-1$^+$ cells, for example, comprises a first step being a solid phase sorting step utilizing for example magnetic activated cell sorting (MACS) recognizing high level expression of STRO-1. A second sorting step can then follow, should that be desired, to result in a higher level of precursor cell expression as described in patent specification WO 01/14268. This second sorting step might involve the use of two or more markers.

The method obtaining STRO-1$^+$ cells might also include the harvesting of a source of the cells before the first enrichment step using known techniques. Thus the tissue will be surgically removed. Cells comprising the source tissue will then be separated into a so called single cells suspension. This separation may be achieved by physical and or enzymatic means.

Once a suitable STRO-1$^+$ cell population has been obtained, it may be cultured or expanded by any suitable means to obtain MEMPs.

In one example, the cells are taken from the subject to be treated, cultured *in vitro* using standard techniques and used to obtain supernatant or soluble factors or expanded cells for administration to the subject as an autologous or allogeneic composition. In an alternative example, cells of one or more of the established human cell lines are used to obtain the supernatant or soluble factors. In another useful example of the disclosure, cells of a non-human animal (or if the patient is not a human, from another species) are used to obtain supernatant or soluble factors.

The disclosure can be practiced using cells from any non-human animal species, including but not limited to non-human primate cells, ungulate, canine, feline, lagomorph, rodent, avian, and fish cells. Primate cells with which the disclosure may be performed include but are not limited to cells of chimpanzees, baboons, cynomolgus monkeys, and any other New or Old World monkeys. Ungulate cells with which the disclosure may be performed include but are not limited to cells of bovines, porcines, ovines, caprines, equines, buffalo and bison. Rodent cells with which the disclosure

may be performed include but are not limited to mouse, rat, guinea pig, hamster and gerbil cells. Examples of lagomorph species with which the disclosure may be performed include domesticated rabbits, jack rabbits, hares, cottontails, snowshoe rabbits, and pikas. Chickens (*Gallus gallus*) are an example of an avian species with which the disclosure may be performed.

Cells useful for the methods of the disclosure may be stored before use, or before obtaining the supernatant or soluble factors. Methods and protocols for preserving and storing of eukaryotic cells, and in particular mammalian cells, are known in the art (cf., for example, Pollard, J. W. and Walker, J. M. (1997) Basic Cell Culture Protocols, Second Edition, Humana Press, Totowa, N.J.; Freshney, R. I. (2000) Culture of Animal Cells, Fourth Edition, Wiley-Liss, Hoboken, N.J.). Any method maintaining the biological activity of the isolated stem cells such as mesenchymal stem/progenitor cells, or progeny thereof, may be utilized in connection with the present disclosure. In one example, the cells are maintained and stored by using cryo-preservation.

Genetically-Modified Cells

In one example, the STRO-1$^+$ cells and/or progeny cells thereof are genetically modified, e.g., to express and/or secrete a protein of interest, e.g., a protein providing a therapeutic and/or prophylactic benefit, e.g., a polypeptide that reduces or prevents T cell activation or that induces proliferation and/or differentiation of neurons and/or myelin production. Exemplary T cell antagonists include, for example, peptides described in Toda *et al., Eur. J. Immunol., 30*: 403-414, 2000.

In another example, the STRO-1$^+$ cells and/or progeny cells thereof are genetically modified to express a protein that treats an inflammatory neurological condition, e.g., beta-interferon.

Methods for genetically modifying a cell will be apparent to the skilled artisan. For example, a nucleic acid that is to be expressed in a cell is operably-linked to a promoter for inducing expression in the cell. For example, the nucleic acid is linked to a promoter operable in a variety of cells of a subject, such as, for example, a viral promoter, e.g., a CMV promoter (e.g., a CMV-IE promoter) or a SV-40 promoter. Additional suitable promoters are known in the art and shall be taken to apply *mutatis mutandis* to the present example of the disclosure.

For example, the nucleic acid is provided in the form of an expression construct. As used herein, the term "expression construct" refers to a nucleic acid that has the ability to confer expression on a nucleic acid (e.g. a reporter gene and/or a counter-

selectable reporter gene) to which it is operably connected, in a cell. Within the context of the present disclosure, it is to be understood that an expression construct may comprise or be a plasmid, bacteriophage, phagemid, cosmid, virus sub-genomic or genomic fragment, or other nucleic acid capable of maintaining and/or replicating heterologous DNA in an expressible format.

Methods for the construction of a suitable expression construct for performance of the disclosure will be apparent to the skilled artisan and are described, for example, in Ausubel et al (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) or Sambrook et al (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001). For example, each of the components of the expression construct is amplified from a suitable template nucleic acid using, for example, PCR and subsequently cloned into a suitable expression construct, such as for example, a plasmid or a phagemid.

Vectors suitable for such an expression construct are known in the art and/or described herein. For example, an expression vector suitable for the method of the present disclosure in a mammalian cell is, for example, a vector of the pcDNA vector suite supplied by Invitrogen, a vector of the pCI vector suite (Promega), a vector of the pCMV vector suite (Clontech), a pM vector (Clontech), a pSI vector (Promega), a VP 16 vector (Clontech) or a vector of the pcDNA vector suite (Invitrogen).

The skilled artisan will be aware of additional vectors and sources of such vectors, such as, for example, Life Technologies Corporation, Clontech or Promega.

Means for introducing the isolated nucleic acid molecule or a gene construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

Alternatively, an expression construct of the disclosure is a viral vector. Suitable viral vectors are known in the art and commercially available. Conventional viral-based systems for the delivery of a nucleic acid and integration of that nucleic acid into a host cell genome include, for example, a retroviral vector, a lentiviral vector or an adeno-associated viral vector. Alternatively, an adenoviral vector is useful for introducing a nucleic acid that remains episomal into a host cell. Viral vectors are an efficient and versatile method of gene transfer in target cells and tissues. Additionally, high

transduction efficiencies have been observed in many different cell types and target tissues.

For example, a retroviral vector generally comprises cis-acting long terminal repeats (LTRs) with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of a vector, which is then used to integrate the expression construct into the target cell to provide long term expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SrV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher *et al., J Virol. 56*:2731-2739 (1992); Johann *et al, J. Virol. 65*:1635-1640 (1992); Sommerfelt *et al, Virol. 76*:58-59 (1990); Wilson *et al, J. Virol. 63*:274-2318 (1989); Miller *et al., J. Virol. 65*:2220-2224 (1991); PCT/US94/05700; Miller and Rosman *BioTechniques 7*:980-990, 1989; Miller, A. D. *Human Gene Therapy 7*:5-14, 1990; Scarpa *et al Virology 75*:849-852, 1991; Burns *et al. Proc. Natl. Acad. Sci USA 90*:8033-8037, 1993).

Various adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors can be readily constructed using techniques known in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski *et al. Molec. Cell. Biol. 5*:3988-3996, 1988; Vincent et al. (1990) Vaccines 90 (Cold Spring Harbor Laboratory Press);Carter *Current Opinion in Biotechnology 5*:533-539, 1992; Muzyczka. *Current Topics in Microbiol, and Immunol. 158*:97-129, 1992; Kotin, Human Gene Therapy 5:793-801, 1994; Shelling and Smith *Gene Therapy 7*:165-169, 1994; and Zhou *et al. J Exp. Med. 179*:1867-1875, 1994.

Additional viral vectors useful for delivering an expression construct of the disclosure include, for example, those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus or an alphavirus or a conjugate virus vector (e.g. that described in Fisher-Hoch *et al., Proc. Natl Acad. Sci. USA 56*:317-321, 1989).

Assaying Therapeutic/Prophylactic Potential of Cells and Soluble Factors

Methods for determining the ability of cells or soluble factors to treat or prevent or delay the onset or progression of an inflammatory neurological condition will be apparent to the skilled artisan.

For example, cells or soluble factors (e.g., a mixture of factors or a single factor or a fraction of factors (e.g., derived by affinity purification or chromatography)) are screened to identify therapeutic agents in *in vitro* models of components of

inflammatory neurological disease (e.g., MS) pathology. Exemplary models include those making use of isolated T cells or mixed lymphocyte populations from transgenic mouse models of MS that comprise a transgenic T cell receptor that reacts with a component of the sheath, e.g., myelin basic protein, myelin oligodendrocyte glycoprotein or myelin proteolipid protein. The cells are contacted with the myelin protein in the presence and absence of cells and/or soluble factors and the level of inflammatory response is assessed, e.g., by detecting secretion of pro-inflammatory cytokines, such as interleukin (IL)-2 or interferon γ. Alternatively, or in addition, proliferative response of cells is assessed, e.g., using (^{3}H)thymidine incorporation. Cells and/or soluble factors that reduce an inflammatory response are selected as a therapeutic. Exemplary assays are described in Illés *et al., Proc. Natl. Acad. Sci. USA,* 101: 11749-11754, 2004 or Rossi *et al., J. Biomolecular Screening, 12*: 481-489, 2007.

Cells and/or soluble factors are also tested in *in vivo* models of inflammatory neurological disease. Exemplary models include EAE models in which a mouse or rat is immunized with a myelin sheath protein or peptide derived therefrom (e.g., MOG, MBP or PLP) and an immune response is generated against the protein thereby inducing a model of MS. Alternatively, T cells that are immunoreactive with a myelin sheath protein are introduced into mice or rats to induce EAE. Exemplary EAE models are reviewed in, for example Tsunoda and Fujinami, *J Neuropathol Exp Neurol.55*:673-686, 1996.

Other models of MS include transgenic animals expressing T cell receptors specific for a myelin protein, e.g., MOG, MBP or PLP. Exemplary models are described, for example, in Bettelli *et al., JEM 197*:1073-1081, 2003; Illés *et al., Proc. Natl. Acad. Sci. USA,* 101: 11749-11754, 2004; or Rossi *et al., J. Biomolecular Screening, 12*: 481-489, 2007; or are commercially available, e.g., from Jackson Laboratories USA (e.g. mice 2D2 having transgenic T cell receptors reactive with MOG).

Exemplary models of SLE that develop inflammatory neurological symptoms include models or anti-phospholipid syndrome (e.g., as described in Ziporen *et al., J. Clin, Invest., 100:* :613-613, 1997) or models reviewed in Brey *et al., Annals NY Acad Sci., 823*: 97-106, 1996.

Models of Guillain-Barré syndrome include those caused by sensitization of animals, e.g., rabbits with ganglioside GM1 (e.g., as described in Yuki *et al., Ann Neurol. 49*: 712-720, 2001).

It will be apparent to the skilled artisan from the foregoing that the present disclosure also provides a method for identifying or isolating a cell or a soluble factor

for the treatment, prevention or delay of an inflammatory neurological condition, said method comprising:

(i) administering a cell or a soluble factor to a test subject suffering from an inflammatory neurological condition and assessing the inflammatory response or neurological function/dysfunction of the subject;

(ii) comparing the inflammatory response or neurological function/dysfunction of the subject at (i) to the inflammatory response or neurological function/dysfunction of a control subject suffering from the inflammatory neurological condition to which the cell or soluble factor has not been administered,

wherein improved inflammatory response or neurological function/dysfunction in the test subject compared to the control subject indicates that the cell or soluble factor treats the inflammatory neurological condition.

The cell may be any cell described herein according to any example.

Cellular Compositions

In one example of the present disclosure STRO-1$^+$ cells and/or progeny cells thereof are administered in the form of a composition. For example, such a composition comprises a pharmaceutically acceptable carrier and/or excipient.

The terms "carrier" and "excipient" refer to compositions of matter that are conventionally used in the art to facilitate the storage, administration, and/or the biological activity of an active compound (see, e.g., Remington's Pharmaceutical Sciences, 16th Ed., Mac Publishing Company (1980). A carrier may also reduce any undesirable side effects of the active compound. A suitable carrier is, for example, stable, e.g., incapable of reacting with other ingredients in the carrier. In one example, the carrier does not produce significant local or systemic adverse effect in recipients at the dosages and concentrations employed for treatment.

Suitable carriers for the present disclosure include those conventionally used, e.g., water, saline, aqueous dextrose, lactose, Ringer's solution, a buffered solution, hyaluronan and glycols are exemplary liquid carriers, particularly (when isotonic) for solutions. Suitable pharmaceutical carriers and excipients include starch, cellulose, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, glycerol, propylene glycol, water, ethanol, and the like.

In another example, a carrier is a media composition, e.g., in which a cell is grown or suspended. For example, such a media composition does not induce any adverse effects in a subject to whom it is administered.

Exemplary carriers and excipients do not adversely affect the viability of a cell and/or the ability of a cell to reduce, prevent or delay an inflammatory neurological condition.

In one example, the carrier or excipient provides a buffering activity to maintain the cells and/or soluble factors at a suitable pH to thereby exert a biological activity, e.g., the carrier or excipient is phosphate buffered saline (PBS). PBS represents an attractive carrier or excipient because it interacts with cells and factors minimally and permits rapid release of the cells and factors, in such a case, the composition of the disclosure may be produced as a liquid for direct application to the blood stream or into a tissue or a region surrounding or adjacent to a tissue, e.g., by injection.

STRO-1$^+$ cells and/or progeny cells thereof can also be incorporated or embedded within scaffolds that are recipient-compatible and which degrade into products that are not harmful to the recipient. These scaffolds provide support and protection for cells that are to be transplanted into the recipient subjects. Natural and/or synthetic biodegradable scaffolds are examples of such scaffolds.

A variety of different scaffolds may be used successfully in the practice of the disclosure. Exemplary scaffolds include, but are not limited to biological, degradable scaffolds. Natural biodegradable scaffolds include collagen, fibronectin, and laminin scaffolds. Suitable synthetic material for a cell transplantation scaffold should be able to support extensive cell growth and cell function. Such scaffolds may also be resorbable. Suitable scaffolds include polyglycolic acid scaffolds, e.g., as described by Vacanti, *et al. J. Ped. Surg. 23*:3-9 1988; Cima, *et al. Biotechnol. Bioeng. 38*:145 1991; Vacanti, *et al. Plast. Reconstr. Surg. 88*:753-9 1991; or synthetic polymers such as polyanhydrides, polyorthoesters, and polylactic acid.

In another example, the cells may be administered in a gel scaffold (such as Gelfoam from Upjohn Company.

The cellular compositions useful for methods described herein may be administered alone or as admixtures with other cells. Cells that may be administered in conjunction with the compositions of the present disclosure include, but are not limited to, other multipotent or pluripotent cells or stem cells, or bone marrow cells. The cells of different types may be admixed with a composition of the disclosure immediately or shortly prior to administration, or they may be co-cultured together for a period of time prior to administration.

In one example, the composition comprises an effective amount or a therapeutically or prophylactically effective amount of cells. For example, the composition comprises about $1x10^5$ STRO-1$^+$ cells/kg to about $1x10^7$ STRO-1$^+$ cells/kg

or about $1x10^6$ STRO-1^+ cells/kg to about $5x10^6$ STRO-1^+ cells/kg. The exact amount of cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the patient, and the extent and severity of the inflammatory neurological condition.

In some examples, cells are contained within a chamber that does not permit the cells to exit into a subject's circulation, however that permits factors secreted by the cells to enter the circulation. In this manner soluble factors may be administered to a subject by permitting the cells to secrete the factors into the subject's circulation. Such a chamber may equally be implanted at a site in a subject to increase local levels of the soluble factors.

In some examples of the disclosure, it may not be necessary or desirable to immunosuppress a patient prior to initiation of therapy with cellular compositions. Accordingly, transplantation with allogeneic, or even xenogeneic, STRO-1^+ cells or progeny thereof may be tolerated in some instances.

However, in other instances it may be desirable or appropriate to pharmacologically immunosuppress a patient prior to initiating cell therapy and/or reduce an immune response of a subject against the cellular composition. This may be accomplished through the use of systemic or local immunosuppressive agents, or it may be accomplished by delivering the cells in an encapsulated device. The cells may be encapsulated in a capsule that is permeable to nutrients and oxygen required by the cell and therapeutic factors the cell is yet impermeable to immune humoral factors and cells. In one example, the encapsulant is hypoallergenic, is easily and stably situated in a target tissue, and provides added protection to the implanted structure. These and other means for reducing or eliminating an immune response to the transplanted cells are known in the art. As an alternative, the cells may be genetically modified to reduce their immunogenicity.

Compositions of Soluble Factors

In one example of the present disclosure, STRO-1^+ cell-derived and/or progeny cell-derived supernatant or soluble factors are administered in the form of a composition, e.g., comprising a suitable carrier and/or excipient. For example, the carrier or excipient does not adversely affect the biological effect of the soluble factors or supernatant.

In one example, the composition comprises a composition of matter to stabilize a soluble factor or a component of supernatant, e.g., a protease inhibitor. For example,

the protease inhibitor is not included in an amount sufficient to have an adverse effect on a subject.

Compositions comprising STRO-1$^+$ cell-derived and/or progeny cell-derived supernatant or soluble factors may be prepared as appropriate liquid suspensions, e.g., in culture medium or in a stable carrier or a buffer solution, e.g., phosphate buffered saline. Suitable carriers are described herein above. In another example, suspensions comprising STRO-1$^+$ cell-derived and/or progeny cell-derived supernatant or soluble factors are oily suspensions for injection. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil; or synthetic fatty acid esters, such as ethyl oleate or triglycerides; or liposomes. Suspensions to be used for injection may also contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Sterile injectable solutions can be prepared by incorporating the supernatant or soluble factors in the required amount in an appropriate solvent with one or a combination of ingredients described above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the supernatant or soluble factors into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, exemplary methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In accordance with an alternative aspect of the disclosure, the supernatant or soluble factors may be formulated with one or more additional compounds that enhance its solubility.

Other exemplary carriers or excipients are described, for example, in Hardman, et al. (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N. Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N. Y.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N. Y.

Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride are included in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the soluble factors may be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

The supernatant or soluble factors may be administered in combination with an appropriate matrix, for instance, to provide slow release of the soluble factors.

Additional Components of Compositions

The STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof may be administered with other beneficial drugs or biological molecules (growth factors, trophic factors). When administered with other agents, they may be administered together in a single pharmaceutical composition, or in separate pharmaceutical compositions, simultaneously or sequentially with the other agents (either before or after administration of the other agents). Bioactive factors which may be co-administered include anti-apoptotic agents (e.g., EPO, EPO mimetibody, TPO, IGF-I and IGF-II, HGF, caspase inhibitors); anti-inflammatory agents (e.g., p38 MAPK inhibitors, TGF-beta inhibitors, statins, IL-6 and IL-1 inhibitors, PEMIROLAST, TRANILAST, REMICADE, SIROLIMUS, and NSAIDs (non-steroidal anti-inflammatory drugs; e.g., TEPOXALIN, TOLMETIN, SUPROFEN); immunosupressive/immunomodulatory agents (e.g., calcineurin inhibitors, such as

cyclosporine, tacrolimus; mTOR inhibitors (e.g., SIROLIMUS, EVEROLIMUS); anti-proliferatives (e.g., azathioprine, mycophenolate mofetil); corticosteroids (e.g., prednisolone, hydrocortisone); antibodies such as monoclonal anti-IL-2Ralpha receptor antibodies (e.g., basiliximab, daclizumab), polyclonal anti-T-cell antibodies (e.g., anti-thymocyte globulin (ATG); anti-lymphocyte globulin (ALG); monoclonal anti-T cell antibody OKT3)); anti-thrombogenic agents (e.g., heparin, heparin derivatives, urokinase, PPack (dextrophenylalanine proline arginine chloromethylketone), antithrombin compounds, platelet receptor antagonists, anti-thrombin antibodies, anti-platelet receptor antibodies, aspirin, dipyridamole, protamine, hirudin, prostaglandin inhibitors, and platelet inhibitors); and anti-oxidants (e.g., probucol, vitamin A, ascorbic acid, tocopherol, coenzyme Q-10, glutathione, L-cysteine, N-acetylcysteine) as well as local anesthetics.

In one example, a composition as described herein according to any example comprises an additional factor for the treatment or prophylaxis of an inflammatory neurological condition.

Alternatively, or in addition, cells, secreted factors and/or a composition as described herein according to any example is combined with a known treatment of an inflammatory neurological condition.

In one example, a pharmaceutical composition as described herein according to any example comprises a compound used to treat an inflammatory neurological condition or a symptom thereof. Alternatively, a method of treatment/prophylaxis as described herein according to any example of the disclosure additionally comprises administering a compound used to treat an inflammatory neurological condition or a symptom thereof. Exemplary compounds include a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody (e.g. an anti-LFA-1 antibody such as efalizumab (RAPTIVA®) commercially available from Genentech, or an anti alpha-4 integrin antibody such as natalizumab (TYSABRI®) available from Biogen Idec/Elan Pharmaceuticals, Inc) etc, or an antibody that binds a B cell surface marker (e.g. anti-CD20 antibody such as rituximab (Rituxan® or Mabthera® or ocrelizumab (both available from Genentech) or ofatumumab (Arzerra®) available from Genmab/Glaxo Group)).

In some examples of combination therapy, the cells, factors and/or composition is/are combined with an interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-lb (BETASERON®); an oligopeptide such a glatiramer acetate (COPAXONE®); a cytotoxic agent such as mitoxantrone (NOVANTRONE®),

methotrexate, cyclophosphamide, chlorambucil, azathioprine; intravenous immunoglobulin (gamma globulin); lymphocyte-depleting therapy (e.g., mitoxantrone, cyclophosphamide, Campath, anti-CD4 antibody, cladribine, total body irradiation, bone marrow transplantation); corticosteroid (e.g. methylprednisolone, prednisone, dexamethasone, or glucorticoid), including systemic corticosteroid therapy; non-lymphocyte -depleting immunosuppressive therapy (e.g., mycophenolate mofetil (MMF) or cyclosporine); cholesterol-lowering drug of the "statin" class, which includes cerivastatin (BAYCOL®), fluvastatin (LESCOL®), atorvastatin (LIPITOR®), lovastatin (MEVACOR®), pravastatin (PRAVACHOL®), Simvastatin (ZOCOR®); estradiol; testosterone (optionally at elevated dosages; Stuve *et al. Neurology 8*:290-301, 2002); hormone replacement therapy; treatment for symptoms secondary or related to MS (e.g., spasticity, incontinence, pain, fatigue); disease -modifying anti-rheumatic drug (DMARD); non-steroidal antiinflammatory drug (NSAID); plasmapheresis; levothyroxine; cyclosporin A; somatastatin analog; cytokine or cytokine receptor antagonist; anti-metabolite; immunosuppressive agent; rehabilitative surgery; radioiodine; or a thyroidectomy.

In another example, a composition as described herein according to any example additionally comprises a factor that induces or enhances differentiation of a progenitor cell into a vascular cell. Exemplary factors include, vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF; e.g., PDGF-BB), and FGF.

In another example, a composition as described herein according to any example additionally comprises a tissue specific committed cell (TSCC). In this respect, International Patent Application No. PCT/AU2005/001445 demonstrates that administration of a TSCC and a STRO-1$^+$ cells can lead to enhanced proliferation of the TSCC. In one example, the TSCC is a neuronal cell, e.g., a neuron, a neuronal progenitor cell or a Schwann cell. Administration of such a composition to a subject may lead to increased production of, for example, neurons or myelin. In another example, the TSCC is a vascular cell. Administration of such a composition to a subject may lead to increased production of vasculature, e.g., leading to increased nutrients being delivered to the affected tissue.

Medical Devices

The present disclosure also provides medical devices for use or when used in a method as described herein according to any example. For example, the present disclosure provides a syringe or catheter or other suitable delivery device comprising STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors therefrom and/or a

composition as described herein according to any example. Optionally, the syringe or catheter is packaged with instructions for use in a method as described herein according to any example.

In another example, the present disclosure provides an implant comprising STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors therefrom and/or a composition as described herein according to any example. Optionally, the implant is packaged with instructions for use in a method as described herein according to any example. Suitable implants may be formed with a scaffold, e.g., as described herein above and STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors therefrom.

Modes of Administration

The STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof may be surgically implanted, injected, delivered (e.g., by way of a catheter or syringe), or otherwise administered directly or indirectly to the site in need of repair or augmentation.

In on example, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof is/are delivered to the blood stream of a subject. For example, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered parenterally. Exemplary routes of parenteral administration include, but are not limited to, intraperitoneal, intraventricular, intracerebroventricular, intrathecal. In one example, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered intra-arterially, into an aorta, into an atrium or ventricle of the heart or into a blood vessel.

In the case of cell delivery to an atrium or ventricle of the heart, cells can be administered to the left atrium or ventricle to avoid complications that may arise from rapid delivery of cells to the lungs.

In one example, the STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are injected into the site of delivery, e.g., using a syringe or through a catheter or a central line.

Selecting an administration regimen for a therapeutic formulation depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, and the immunogenicity of the entity. For example, an administration regimen maximizes the amount of therapeutic compound delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of formulation delivered depends in part on the particular entity and the severity of the condition being treated.

In one example, STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are delivered as a single bolus dose. Alternatively, STRO-1$^+$ cell-derived supernatant or soluble factors, STRO-1$^+$ cells or progeny thereof are administered by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. An exemplary dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose depends on the type and activity of the compound being used. Determination of the appropriate dose is made by a clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects.

In accordance with examples of the disclosure directed to treating or delaying the progression of an inflammatory neurological condition, the STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom can be administered following diagnosis of the disorder, e.g., using standard methods known in the art and/or described herein.

For those examples directed to preventing or delaying the onset of an inflammatory neurological condition, the STRO-1$^+$ cells and/or progeny cells thereof and/or soluble factors derived therefrom can administered prior to clinical diagnosis of the disorder, e.g., when the subject has suffered from a lesion in myelin however is yet to be diagnosed with MS and/or has produced antiphospholipid antibodies.

The present disclosure is described further in the following non-limiting examples.

EXAMPLES

Example 1: Immunoselection of MPCs by Selection of STRO-3$^+$ Cells

Bone marrow (BM) is harvested from healthy normal adult volunteers (20-35 years old). Briefly, 40 ml of BM is aspirated from the posterior iliac crest into lithium-heparin anticoagulant-containing tubes.

BMMNC are prepared by density gradient separation using Lymphoprep™ (Nycomed Pharma, Oslo, Norway) as previously described (Zannettino, A.C. *et al.* (1998) *Blood* 92: 2613-2628). Following centrifugation at 400 x g for 30 minutes at 4°C, the buffy layer is removed with a transfer pipette and washed three times in "HHF", composed of Hank's balanced salt solution (HBSS; Life Technologies,

Gaithersburg, MD), containing 5% fetal calf serum (FCS, CSL Limited, Victoria, Australia).

STRO-3$^+$ (or TNAP$^+$) cells were subsequently isolated by magnetic activated cell sorting as previously described (Gronthos *et al.* (2003) *Journal of Cell Science 116*: 1827-1835; Gronthos, S. and Simmons, P.J. (1995) *Blood* 85: 929-940). Briefly, approximately 1-3 x 10^8 BMMNC are incubated in blocking buffer, consisting of 10% (v/v) normal rabbit serum in HHF for 20 minutes on ice. The cells are incubated with 200µl of a 10µg/ml solution of STRO-3 mAb in blocking buffer for 1 hour on ice. The cells are subsequently washed twice in HHF by centrifugation at 400 x g. A 1/50 dilution of goat anti-mouse γ-biotin (Southern Biotechnology Associates, Birmingham, UK) in HHF buffer is added and the cells incubated for 1 hour on ice. Cells are washed twice in MACS buffer (Ca^{2+} - and Mn^{2+} -free PBS supplemented with 1% BSA, 5 mM EDTA and 0.01% sodium azide) as above and resuspended in a final volume of 0.9 ml MACS buffer.

One hundred µl streptavidin microbeads (Miltenyi Biotec; Bergisch Gladbach, Germany) are added to the cell suspension and incubated on ice for 15 minutes. The cell suspension is washed twice and resuspended in 0.5 ml of MACS buffer and subsequently loaded onto a mini MACS column (MS Columns, Miltenyi Biotec), and washed three times with 0.5 ml MACS buffer to retrieve the cells which did not bind the STRO-3 mAb (deposited on 19 December 2005 with American Type Culture Collection (ATCC) under accession number PTA-7282 - see International Publication No.WO 2006/108229). After addition of a further 1 ml MACS buffer, the column is removed from the magnet and the TNAP$^+$ cells are isolated by positive pressure. An aliquot of cells from each fraction can be stained with streptavidin-FITC and the purity assessed by flow cytometry.

Example 2: Cells Selected by STRO-3 mAb are STRO-1^{bright} Cells

Experiments were designed to confirm the potential of using STRO-3 mAb as a single reagent for isolating cells STRO-1^{bright} cells.

Given that STRO-3 (IgG1) is a different isotype to that of STRO-1 (IgM), the ability of STRO-3 to identify clonogenic CFU-F was assessed by two-color FACS analysis based on its co-expression with STRO-1$^+$ cells isolated using the MACS procedure (Figure 1). The dot plot histogram represents 5 x 10^4 events collected as listmode data. The vertical and horizontal lines were set to the reactivity levels of <1.0% mean fluorescence obtained with the isotype-matched control antibodies, 1B5 (IgG) and 1A6.12 (IgM) treated under the same conditions. The results demonstrate

that a minor population of STRO-1^{bright} cells co-expressed TNAP (upper right quadrant) while the remaining STRO-1^{+} cells failed to react with the STRO-3 mAb. Cells isolated by FACS from all four quadrants were subsequently assayed for the incidence of CFU-F (Table 1).

Table 1: Enrichment of human bone marrow cells by dual-colour FACS analysis based on the co-expression of the cell surface markers STRO-1 and TNAP (refer to Figure 1). FACS sorted cells were cultured under standard clonogenic conditions in alpha MEM supplemented with 20% FCS. The data represents the mean number of day 14 colony-forming cells (CFU-F) per 10^5 cells plated ± SE (n=3 different bone marrow aspirates). These data suggest that human MPC are exclusively restricted to the TNAP positive fraction of BM which co-express the STRO-1 antigen brightly.

Bone Marrow Fraction	Frequency of CFU-F/10^5 Cells	Enrichment (Fold Increase)
Unfractionated BMMNC	11.0 ± 2.2	1.0
TNAP^{+}/STRO-1^{bright}	4,511 ± 185	410
TNAP^{+}/STRO-1^{dull}	0.0	0.0

Example 3: Relative Gene and Surface Protein Expression of STRO-1^{dull} and STRO-1^{bri} Cells

In the first series of experiments, semi-quantitative RT-PCR analysis was employed to examine the gene expression profile of various lineage-associated genes expressed by STRO-1^{dull} or STRO-1^{bri} populations, isolated by fluorescence activated cell sorting (Figure 2A). In the second series of experiments, flow cytometry and mean channel fluorescence analysis was employed to examine the surface protein expression profile of various lineage-associated proteins expressed by STRO-1^{dull} or STRO-1^{bri} populations, isolated by fluorescence activated cell sorting.

Total cellular RNA was prepared from either 2 x 10^6 STRO-1^{bri} or STRO-1^{dull} sorted primary cells, chondrocyte pellets and other induced cultures and lysed using RNAzolB extraction method (Biotecx Lab. Inc., Houston, TX), according to the manufacturer's recommendations. RNA isolated from each subpopulation was then used as a template for cDNA synthesis, prepared using a First-strand cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden). The expression of various transcripts was assessed by PCR amplification, using a standard protocol as described previously (Gronthos *et al., J. Bone and Min. Res. 14*:48-57, 1999). Primer sets used in this study are shown in Table 2. Following amplification, each reaction mixture was analyzed by

1.5% agarose gel electrophoresis, and visualized by ethidium bromide staining. RNA integrity was assessed by the expression of GAPDH.

Relative gene expression for each cell marker was assessed with reference to the expression of the house-keeping gene, GAPDH, using ImageQant software (Figure 2B, C). In addition, dual-colour flow cytometric analysis was used to examine the protein expression profile of *ex vivo* expanded MPC based on their expression of a wider range of cell lineage-associated markers in combination with the STRO-1 antibody. A summary of the general phenotype based on the gene and protein expression of STRO-1^{dull} and STRO-1^{bri} cultured cells is presented in Table 3. The data indicate that *ex vivo* expanded STRO-1^{bri} MPC exhibit differentially higher expression of markers associated with perivascular cells, including angiopoietin-1, VCAM-1, SDF-1, IL-1_{β}, TNFα, and RANKL. Comparisons between the protein and gene expression profiles of STRO-1^{dull} and STRO-1^{bri} cultured cells are summarized in Tables 3 and 4.

Subtractive hybridization studies were also performed in order to identify genes uniquely expressed by STRO-1^{bri} cells. Briefly, STRO-1^{dull} and STRO-1^{bri} were isolated as described above (see Figure 3A). Total RNA was prepared from STRO-1^{dull} and STRO-1^{bri} cells pooled from 5 different marrow samples using the RNA STAT-60 system (TEL-TEST). First-strand synthesize was performed using the SMART cDNA synthesis kit (Clontech Laboratories). The resultant mRNA/single-stranded cDNA hybrid was amplified by long-distance PCR (Advantage 2 PCR kit; Clontech) using specific primer sites at the 3' and 5' prime ends formed during the initial RT process according to the manufacturer's specifications. Following RsaI digestion of the STRO-1bright cDNA, 2 aliquots were used to ligate different specific adaptor oligonucleotides using the Clontech PCR-Select cDNA Subtraction Kit. Two rounds of subtractive hybridization were performed using STRO-1^{bri} (tester) and STRO-1^{dull} (driver) cDNA, and vice versa, according to the manufacturer's protocol. This procedure was also performed in reverse using STRO-1^{dull} tester cDNA hybridized against STRO-1^{bri} driver cDNA.

To identify genes uniquely expressed by STRO-1^{bri} population, STRO-1^{bri}–subtracted cDNA was used to construct replicate low-density microarray filters comprising 200 randomly selected bacterial clones transformed with the STRO-1^{bri}subtracted cDNAs ligated into a T/A cloning vector. The microarrays were subsequently probed with either [^{32}P] dCTP–labeled STRO-1^{bri} or STRO-1^{dull} subtracted cDNA (Figure 3B-C). Differential screening identified a total of 44 clones, which were highly differentially expressed between the STRO-1^{dull} and STRO-1^{bright} subpopulations. DNA sequencing of all the differentially expressed clones revealed

that only 1 clone was representative of a known stromal cell mitogen; namely, platelet-derived growth factor (PDGF) (Gronthos and Simmons, *Blood. 85*: 929-940, 1995). Interestingly, 6 of the 44 clones were found to contain DNA inserts corresponding to the chemokine, stromal-derived factor-1 (SDF-1). The high abundance of SDF-1 transcripts in human STRO-1^{bright} cells was confirmed by semiquantitative RT-PCR of total RNA prepared from freshly sorted STRO-1^{bright}, STRO-1^{dull}, and STRO-1^{negative} bone marrow subpopulations (Figure 3D and Table 3).

Table 2. RT-PCR primers and conditions for the specific amplification of human mRNA

Target Gene	Sense/ Antisense (5'-3') Primer Sequences	Product Size
GAPDH	CACTGACACGTTGGCAGTGG (SEQ ID NO: 1) CATGGAGAAGGCTGGGGCTC (SEQ ID NO: 2)	417
SDF-1	GAGACCCGCGCTCGTCCGCC (SEQ ID NO: 3) GCTGGACTCCTACTGTAAGGG (SEQ ID NO: 4)	364
IL-1β	AGGAAGATGCTGGTTCCCTCTC (SEQ ID NO: 5) CAGTTCAGTGATCGTACAGGTGC (SEQ ID NO: 6)	151
FLT-1	TCACTATGGAAGATCTGATTTCTTACAGT (SEQ ID NO: 7) GGTATAAATACACATGTGCTTCTAG (SEQ ID NO: 8)	380
TNF-α	TCAGATCATCTTCTCGAACC (SEQ ID NO: 9) CAGATAGATGGGCTCATACC (SEQ ID NO: 10)	361
KDR	TATAGATGGTGTAACCCGGA (SEQ ID NO: 11) TTTGTCACTGAGACAGCTTGG (SEQ ID NO: 12)	450
RANKL	AACAGGCCTTTCAAGGAGCTG (SEQ ID NO: 13) TAAGGAGGGGTTGGAGACCTCG (SEQ ID NO: 14)	538
Leptin	ATGCATTGGGAACCCTGTGC (SEQ ID NO: 15) GCACCCAGGGCTGAGGTCCA (SEQ ID NO: 16)	492
CBFA-1	GTGGACGAGGCAAGAGTTTCA (SEQ ID NO: 17) TGGCAGGTAGGTGTGGTAGTG (SEQ ID NO: 18)	632

Target Gene	Sense/ Antisense (5'-3') Primer Sequences	Product Size
PPARγ2	AACTGCGGGGAAACTTGGGAGATTCTCC (SEQ ID NO: 18) AATAATAAGGTGGAGATGCAGGCTCC (SEQ ID NO: 19)	341
OCN	ATGAGAGCCCTCACACTCCTC (SEQ ID NO: 20) CGTAGAAGCGCCGATAGGC (SEQ ID NO: 21)	289
MyoD	AAGCGCCATCTCTTGAGGTA (SEQ ID NO: 22) GCGAGAAACGTGAACCTAGC (SEQ ID NO: 23)	270
SMMHC	CTGGGCAACGTAGTAAAACC (SEQ ID NO: 24) TATAGCTCATTGCAGCCTCG (SEQ ID NO: 25)	150
GFAP	CTGTTGCCAGAGATGGAGGTT (SEQ ID NO: 26) TCATCGCTCAGGAGGTCCTT (SEQ ID NO: 27)	370
Nestin	GGCAGCGTTGGAACAGAGGTTGGA (SEQ ID NO: 28) CTCTAAACTGGAGTGGTCAGGGCT (SEQ ID NO: 29)	460
SOX9	CTCTGCCTGTTTGGACTTTGT (SEQ ID NO: 30) CCTTTGCTTGCCTTTTACCTC (SEQ ID NO: 31)	598
Collagen type X	AGCCAGGGTTGCCAGGACCA (SEQ ID NO: 32) TTTTCCCACTCCAGGAGGGC (SEQ ID NO: 33)	387
Aggrecan	CACTGTTACCGCCACTTCCC (SEQ ID NO: 34) ACCAGCGGAAGTCCCCTTCG (SEQ ID NO: 35)	184

Table 3. Summary of the Relative Gene Expression in STRO-1^{Bri} and STRO-1^{Dull} populations. A list of genes which displayed measurable and differential expression between the STRO-1^{Bri} and STRO-1^{Dull} populations as determined by reverse transcription-PCR are presented . Values represent the relative gene expression with reference to the house-keeping gene, GAPDH.

		Gene Expression relative to GAPDH	
Tissue	**Marker**	**STRO-1^{Bri}**	**STRO-1^{Dull}**
Neurons	*GFAP (Glial Fibrillary Acidic Protein)*	0.1	0.7
Bone	*OCN (Osteocalcin)*	1.1	2.5
	OSX (Osterix)	0.4	1.3
	CBFA-1 (Core Factor Binding Protein-1)	0.3	0.6
Immunoregulatory	*RANKL (Receptor Activator of Nuclear Factor κ B)*	1.6	0.3
	SDF-1-alpha (Stromal Derived factor-1-alpha)	3.2	0.1
Fat	*Leptin*	3.1	4.2
Cardiomyocytes	*GATA-4*	1.1	2.9
Endothelial cells	*Ang-1 (Angiopoietin-1)*	1.5	0.8
Chondrocytes	*Sox 9*	0.3	1.1
	COL X (Collagen X)	3.5	2.8
Pro-inflammatory Cytokines	*TNF-alpha (Tumor necrosis alpha)*	1.7	0.9

To correlate protein surface expression with density of STRO-1 expression, single cell suspensions of *ex vivo* expanded cells derived bone marrow MPC were prepared by trypsin/EDTA detachment and subsequently incubated with the STRO-1 antibody in combination with antibodies identifying a wide range of cell lineage-associated markers. STRO-1 was identified using a goat anti-murine IgM-fluorescein isothiocyanate while all other markers were identified using either a goat anti-mouse or anti-rabbit IgG- phycoerythrin. For those antibodies identifying intracellular antigens, cell preparations were first labeled with the STRO-1 antibody, fixed with cold 70% ethanol to permeabilize the cellular membrane and then incubated with intracellular antigen-specific antibodies. Isotype matched control antibodies were used under identical conditions. Dual-colour flow cytometric analysis was performed using a COULTER EPICS flow cytometer and list mode data collected. The dot plots represent 5,000 listmode events indicating the level of fluorescence intensity for each lineage cell marker (y-axis) and STRO-1 (x-axis). The vertical and horizontal

quadrants were established with reference to the isotype matched negative control antibodies.

Table 4. Summary of the Relative Protein Expression in STRO-1^{Bri} and STRO-1^{Dull} populations. A list of proteins which displayed differential expression between the STRO-1^{Bri} and STRO-1^{Dull} populations as determined by flow cytometry are presented. Values represent the relative mean fluorescence intensity of staining.

		Mean Fluorescence Intensity	
Tissue	**Marker**	**STRO-1^{Bri}**	**STRO-1^{Dull}**
Neurons	*Neurofilament*	1.7	20.5
Bone	*ALK PHOS (Alkaline Phophatase)*	5.7	44.5
Immunoregulatory	*RANKL (Receptor Activator of Nuclear Factor κ B)*	658.5	31.0
Epithelial Cells	*CytoKeratin 10+13*	1.2	23.3
	Cytokeratin 14	1.8	8.8
Smooth Muscle	*α-SMA (Alpha Smooth Muscle Actin)*	318.0	286.0
Chondrocytes	*Byglycan*	84.4	65.9
Basal Fibroblast	*Tenascin C*	22.2	6.9
Cardiomyocyte	*Troponin C*	2.5	15.0

These results show that SDF-1alpha and RANKL are highly expressed by STRO-1^{bri} cells. This is important because both of these proteins are known to be involved in up-regulation of CD4$^+$ CD25$^+$ regulatory T (Treg) cells which confer protection against immune disorders such as EAE (Loser *et al.*, Nature Medicine 12:1372-1379, 2006; Hess, Biol. Blood Marrow Transplant, 12 (1 Suppl 2):13-21, 2006; and Meiron *et al.*, J. Exp. Medicine 205:2643-2655, 2008).

Example 4 – Effect of STRO-1$^+$ Cells in EAE

For the following experiments the myelin oligodendrocyte glycoprotein (MOG)-induced experimental inflammatory encephalomyelitis (EAE) in C57Bl/6J mice was used. C57Bl/6J mice display similar phenotypic symptoms (progressive paralysis) to that of MS patients as well as showing extensive inflammation, demyelination and axonal loss/damage in the CNS. The immunization procedure for the induction of EAE, assessment of clinical symptoms and MPC transplantation used is as follows.

Active Induction of EAE

Mice were immunized with 200µg recombinant MOG dissolved in Phosphate Buffered Saline (PBS) and mixed with an equal volume of Freund's complete adjuvant containing 400 µg of killed Mycobacterium tuberculosis H37Ra. 0.1 ml of this mixture was injected subcutaneously into the right and left flank (total 0.2 ml/mouse) using a 25 gauge (G) needle. Mice were also immunized with 350ng inactivated *Bordetella* pertussis toxin in 0.30 ml of PBS intravenously (i.v.) via tail vein of on day 0 and day 2 using a 29 G needle. Gentle pressure was applied to the I.V. site for 30 sec after the injection to reduce the risk of bleeding from the i.v. site.

Mice were monitored every 2-5 minutes for 10-15 minutes to ensure there is no active bleeding.

Treatment with MPCs

MPCs were isolated essentially as described in Example 1. On days 8, 10 and 12 after disease induction, $2x10^5$ or $4x10^5$ MPCs were administered as a single intravenous (i.v.) injection in a volume of 200µl PBS (see Table 5). Controls received i.v. injections of equal volumes of PBS only. Mice were monitored daily and clinical signs scored according to the scale described below. Experiments were continued for approximately 36 days to monitor the course of disease. At termination of the experiment, brain, spinal cord and optic nerve were dissected and fixed in formalin solution.

Table 5: Summary of Treatment Regimen

Treatment	**No of cells per mouse per injection**	**Total MPC injected per 20g mouse**	**Number of mice**
PBS I.V.	-	-	12
High dose MPC I.V.	$4 x10^5$ MPC	$6x10^6$MPC/Kg	5
Low dose MPC I.V.	$2x10^5$ MPC	$3x10^6$MPC/Kg	5

Monitoring of Mice

All mice were examined daily for signs of neurological dysfunction for the entirety of the experiment.

Grading of neurological dysfunction:

0 – normal

1 – loss of tail tone only

2 – mild weakness of 1 or 2 hind limbs and abnormal gait

3 – inability to move hind limbs

4 – inability to move hind limbs and mild forelimb weakness

5 – dead

Results

Control C57Bl/6J mice display similar phenotypic symptoms (progressive paralysis) to that of MS patients as well as showing extensive inflammation, demyelination and axonal loss/damage in the CNS.

As shown in Figure 4, intravenously administered MPCs administered at the onset of EAE disease induction are able to inhibit the severity of the mean clinical disease scores over the course of 36 days compared to EAE animals treated with PBS.

Figure 5 shows that MPC treatment induces a dose-dependent reduction in cumulative disease index in chronic progressive EAE (total area under the curve analysis of mean clinical disease score).

The effects of administration of the MPCs are summarized in Table 6:

Table 6: Summary of clinical outcome in mouse EAE model following treatment with MPCs

	PBS	**Total MPC dose 0.6×10^5 (3×10^6 MPC/kg)**	**Total MPC dose 1.2×10^5 (6×10^6 MPC/kg)**
Disease incidence	12/12	5/5	5/5
Day disease onset (range)	13.92 ± 0.54 (11-18)	11.6 ± 0.6 (10-13)	13.8 ± 0.97 (12-17)
Death or severe disease (%)	3/12 (25)	0/5 (0)	0/5 (0)
Maximum clinical score	3.5 ± 0.26	3	2.6 ± 0.4
Cumulative disease index (Area under curve)	62 ± 5.8	55.7 ± 2.1	44.3 ± 4.4

Data in Table 6 show that all animals demonstrate neurological disease between 10-18 days following induction of EAE with MOG peptide 35-55. 25% (3/12) of control animals treated with PBS died in comparison to 0/15 animals treated with MPC The maximum clinical score was the highest in the control group and all MPC treated groups showed a lower maximal clinical score

The cumulative disease index which is the area under the curve (AUC) for the mean clinical score for the duration of 36 days were all lower for the MPC therapy groups compared to that observed for the control group indicating a robust and sustained EAE disease suppression by MPC.

These data show that in this model of a human inflammatory neurological condition human MPCs are effective in reducing the clinical severity of EAE.

Example 4 – Effect of MPCs on T cell Proliferation

MPC-treated mice and controls as described in Example 3 were culled on day 36 after disease induction (MOG35-55 immunization). Splenocytes were cultured *in vitro* with media alone or re-stimulated with MOG_{35-55} and then T-cell proliferative responses were measured through [^{3}H]-thymidine incorporation. The specific

proliferative responses to MOG were compared to the matched splenocytes cultured in media-alone (unstimulated). Splenocytes cultured in PMA/Ionomycin served to determine the non-specific (antigen-independent) stimulation of T cell proliferation.

Data presented in Figure 6 demonstrate that T cell immune responses to secondary *in vitro* antigenic challenge with MOG are inhibited in comparison to T cells cultured from control animals. Data in Figure 7 show that T cell immune responses in animals previously treated with MPC *in vivo* maintain potent responses to non-specific stimulation with PMA/ionomycin in vitro in comparison to T cells cultured from PBS-treated control animals. This exaggerated response to non-specific stimulation may reflect the xenogenic response to human antigens by mouse T-cells.

These data show that human MPCs reduce or prevent T cell immune response to a specific antigen (e.g., antigenic stimulation by MOG), even 24 days after the last administration of MPCs. The data indicate that STRO-1 enriched MPC induce tolerance to multiple sclerosis antigens.

Example 6 - *In Vitro* Effects of MPCs

The immunoregulatory properties of MPC are tested by proliferation assays, mixed lymphocyte reactions and cytokines production as described below.

Proliferation assays and mixed lymphocyte reactions

Mononuclear cells are collected from the spleens of healthy C57BL/6 mice, 2D2 transgenic mice or MOG-immunized mice treated with MPCs or vehicle alone essentially as described in Example 4. Single cell suspensions are prepared in complete RPMI media containing 10% FBS, 2mM L-glutamine, 100units/ml penicillin, 100μg/ml streptomycin (all from Invitrogen), 1mM sodium pyruvate (Sigma) and 50μM β-mercaptoethanol (Sigma). Following red blood cell lysis, cells are washed twice and then seeded in 96-well flat bottom microtiter plates (Nunc) in triplicate at a concentration of 2.5×10^5 cells per well in the presence of either 20μg/ml MOG_{35-55} (GL Biochem), 800ng/ml ionomycin and 20pg/ml phorbol myristate acetate (PMA) (both from Sigma), or into wells pre-coated with 10μg/ml anti-CD3 and 10μg/ml anti-CD8 (both from BD). Cells are then incubated at 37°C with 5%CO_2 for 72 hours and 1μCi/well [3H] thymidine is added during the last 18 hours of culture. Cells are harvested onto filter mats and incorporated radioactive nucleic acids counted on a Top Count Harvester (Packard Biosciences). For experiments involving inhibition of T-cell proliferation by MPC, concentrations of MPC ranging from 2.5 to 0.002×10^4 cells per well are seeded prior to the addition of splenocytes.

In mixed lymphocyte reactions (MLR), 2 x 10^5 splenocytes from C57BL/6 mice (responders) are incubated with equal numbers of irradiated (20Gy) Balb/c stimulators or irradiated MPC and cultured for a period of 5 days, with the addition of 1μCi/well [3H] thymidine during the last 24 hours of culture.

In MLRs involving T-cell inhibition, 2 x 10^4 irradiated MPC are seeded into the wells prior to the addition of splenocytes.

Cytokine production

Supernatants used for analysis of cytokine production are obtained from two day co-cultures of 2.5 x 10^6 splenocytes from 2D2 transgenic mice stimulated with 20μg/ml MOG_{35-55} alone or in the presence of 2 x 10^4 MPC (MPC: splenocyte ratio of 1:10). Quantitative analysis of cytokines us performed using a mouse Th1/Th2/Th17 cytometric bead array (CBA) kit (BD) essentially according to the manufacturer's instructions and analyzed on a BD FACSCanto II flow cytometer. The following cytokines are measured: interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α).

CLAIMS:

1. A method for treating or preventing an inflammatory neurological disease, the method comprising administering to the subject a population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom.

2. The method of claim 1, wherein the inflammatory neurological disease is associated with or caused by a T cell response to an inflammatory stimulus.

3. The method of claim 1 or 2 comprising administering a population of cells enriched for STRO-1^{bright} cells and/or progeny thereof and/or soluble factors derived therefrom.

4. The method of any one of claims 1 to 3, wherein the inflammatory neurological disease is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, Guillain-Barre syndrome, Lambert-Eaton myasthenic syndrome, myasthenia gravis, transverse myelitis, leukodystrophy and progressive multifocal leukoencephalopathy.

5. The method of any one of claims 1 to 4, wherein the disease is systemic lupus erythematosus.

6. The method of any one of claims 1 to 4, wherein the disease is multiple sclerosis.

7. The method of claim 6, wherein the disease is a chronic progressive form of multiple sclerosis or a relapsing-remitting form of multiple sclerosis.

8. The method of any one of claims 1 to 7, wherein the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered systemically.

9. The method of any one of claims 1 to 8, wherein the disease is a relapsing-remitting disease and the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered during disease relapse to prevent or delay relapse of the disease.

10. The method of any one of claims 1 to 9, comprising administering an amount of the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom effective to increase the number of regulatory T (Treg) cells in the subject and/or at the site of pathogenesis of the disease.

11. The method of any one of claims 1 to 10, comprising administering between 2 x 10^6 to 8 x 10^6 STRO-1$^+$ cells and/or progeny thereof per kg.

12. The method of any one of claims 1 to 11, comprising administering between 3 x 10^6 to 6 x 10^6 STRO-1$^+$ cells and/or progeny thereof per kg.

13. The method of any one of claims 1 to 12 comprising administering a low dose of STRO-1$^+$ cells and/or progeny thereof.

14. The method of claim 13, wherein the low dose of STRO-1$^+$ cells and/or progeny thereof comprises between 0.1 x 10^6 and 3 x 10^6 STRO-1$^+$ cells and/or progeny thereof per kg.

15. The method of claim 14, wherein the low dose of STRO-1$^+$ cells and/or progeny thereof comprises about 3 x 10^6 STRO-1$^+$ cells and/or progeny thereof per kg.

16. The method of any one of claims 1 to 15, wherein the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered once weekly or less often.

17. The method of any one of claims 1 to 15, wherein the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom are administered once every four weeks or less often.

18. The method of any one of claims 1 to 17, wherein the population enriched for STRO-1$^+$ cells and/or progeny thereof are genetically-engineered to express a molecule to block stimulation of T cells and/or the soluble factors are from such genetically-modified cells.

19. The method of any one of claims 1 to 18, wherein the population enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors therefrom are administered with a compound to block stimulation of T cells.

20. The method of any one of claims 1 to 19, wherein the population enriched for STRO-1$^+$ cells and/or progeny cells are autogeneic or allogeneic and/or the soluble factors are derived from autogeneic or allogeneic cells.

21. The method of any one of claims 1 to 19, wherein the population enriched for STRO-1$^+$ cells and/or progeny cells have been culture expanded prior to administration and/or prior to obtaining the soluble factors.

22. A method for preventing an immune response in response to an antigen, the method comprising administering to the subject a population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom.

23. The method of claim 22, wherein the immune response is a T cell-mediated immune response.

24. The method of claim 23, wherein the T cell-mediated immune response comprises T cell proliferation.

25. The method of any one of claims 22 to 24, wherein the T cell-mediated immune response is suppressed in response to a specific antigen and a T cell-mediated immune response in response to another antigen is not suppressed.

26. The method of any one of claims 22 to 25, wherein the subject has previously raised an immune response to the antigen and the population, progeny and/or soluble factors suppress a further immune response to the antigen.

27. The method of claim 26, comprising administering the population, progeny and/or soluble factors after the subject raises an immune response to the antigen to thereby prevent a further immune response to the antigen.

28. The method of any one of claims 22 to 27, wherein the immune response is suppressed for at least about 24 days following administration of the population of cells

enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom.

29. A method for inducing tolerance to an antigen in a subject, the method comprising administering to the subject a population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom.

30. The method of any one of claims 22 to 29, wherein the antigen or the specific antigen is one against which an inflammatory response is raised.

31. The method of claim 30, wherein the inflammatory response is causative of an inflammatory neurological disease.

32. The method of any one of claims 1 to 31, wherein the population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom is administered with a compound that treats or prevents an inflammatory neurological disease.

33. The method of claim 32, wherein the compound is glatiramer acetate and/or beta-interferon.

34. A population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom for use in the treatment or prevention of an inflammatory neurological disease and/or for suppressing an immune response against an antigen and/or for inducing tolerance to an antigen..

35. Use of a population of cells enriched for STRO-1$^+$ cells and/or progeny thereof and/or soluble factors derived therefrom in the manufacture of a medicament for treating or preventing an inflammatory neurological disease and/or for suppressing an immune response against an antigen and/or for inducing tolerance to an antigen.