Title: MULTIPLE SCLEROSIS-RELATED IMMUNOGLOBULIN DERIVED PROTEINS, COMPOSITIONS, METHODS AND USES

Abstract: The present invention relates to at least one novel multiple sclerosis related human Ig derived protein or specified portion or variant, including isolated nucleic acids that encode at least one multiple sclerosis related Ig derived protein or specified portion or variant, multiple sclerosis related Ig derived protein or specified portion or variants, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.
MULTIPLE SCLEROSIS-RELATED IMMUNOGLOBULIN DERIVED PROTEINS,
COMPOSITIONS, METHODS AND USES

BACKGROUND OF THE INVENTION

RELATED APPLICATIONS
This application claims priority to US Provisional patent Appl. No. 60/367,896, filed March 26, 2002, which is entirely incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to human Ig derived proteins (Ig derived proteins), specified portions or variants specific for at least one multiple sclerosis related protein or fragment, multiple sclerosis-related immunoglobulin derived protein encoding and complementary nucleic acids, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

RELATED ART

Interleukin-12 (IL-12) is a heterodimeric cytokine consisting of glycosylated polypeptide chains of 35kD (p35) and 40 kD(p40) which are disulfide bonded. The cytokine is synthesized and secreted by antigen presenting cells including dendritic cells, monocytes, macrophages, B cells, Langerhans cells and keratinocytes as well as natural killer (NK) cells. IL-12 mediates a variety of biological processes and has been referred to as NK cell stimulatory factor (NKSF), T-cell stimulating factor, cytotoxic T-lymphocyte maturation factor and EBV-transformed B-cell line factor.

Interleukin-12 can bind to the IL-12 receptor expressed on the plasma membrane of cells (e.g., T cells, NK cell), thereby altering (e.g., initiating, preventing) biological processes. For example, the binding of IL-12 to the IL-12 receptor can stimulate the proliferation of pre-activated T cells and NK cells, enhance the cytolytic activity of cytotoxic T cells (CTL), NK cells and LAK (lymphokine activated killer) cells, induce production of gamma interferon (IFN GAMMA) by T cells and NK cells and induce differentiation of naive Th0 cells into Th1 cells that produce IFN GAMMA and IL-2. In particular, IL-12 is vital for the generation of cytolytic cells (e.g., NK, CTL) and for mounting a cellular immune response (e.g., a Th1 cell mediated immune response). Thus, IL-12 is critically important in the generation and regulation of both protective immunity (e.g., eradication of infections) and pathological immune responses (e.g., autoimmunity). Accordingly, an immune response (e.g., protective or pathogenic) can be enhanced, suppressed or prevented by manipulation of the biological activity of IL-12 in vivo, for example, by means of an antibody.
Interleukin-23 (IL-23) is the name given to a factor that is composed of the p40 subunit of IL12 (IL12beta, IL12-p40) and another protein of 19 kDa, designated p19. p19 is structurally related to IL6, G-CSF, and the p35 subunit of IL12. p19 by itself is biologically inactive while the complex of p19 with p40 is active. The active complex is secreted by dendritic cells after cell activation. Mouse memory T-cells (CD4 (+)CD45 Rb(low)) proliferate in response to IL23 but not in response to IL12.

Human IL23 has been shown to stimulate the production of IFN-gamma by PHA blast T-cells and memory T-cells. It also induces proliferation of both cell types. Human monocyte-derived macrophages produce IL23 in response to virus infection (Sendai virus but not Influenza A virus).

IL23 binds to the beta-1 subunit but not to the beta-2 subunit of the IL12 receptor, activating one of the STAT proteins, STAT-4, in PHA blast T-cells. Parham et al demonstrate that the IL23 receptor consists of a receptor chain, termed IL23R, and the beta-1 subunit of the IL12 receptor. The human IL23R gene is on human chromosome 1 within 150 kb of the gene encoding IL12Rbeta2. IL23 activates the same signaling molecules as IL12: JAK2, Tyk2, and STAT-1, STAT-3, STAT-4, and STAT-5. STAT-4 activation is substantially weaker and different DNA-binding STAT complexes form in response to IL23 compared with IL12. IL23R associates constitutively with JAK2 and in a ligand-dependent manner with STAT-3.

Expression of p19 in transgenic mice leads to runting, systemic inflammation, infertility, and death before 3 months of age. The animals show high serum concentrations of the pro-inflammatory cytokines TNF-alpha and IL1. The number of circulating neutrophils is increased. Acute phase proteins are expressed constitutively. Animals expressing p19 specifically in the liver do not show these abnormalities. Expression of p19 is most likely due to hematopoietic cells as bone marrow transplantation of cells expressing p19 causes the same phenotype as that observed in the transgenic animals.

Non-human, chimeric, polyclonal (e.g., anti-sera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion products thereof) are potential therapeutic agents that are being developed in some cases to attempt to treat certain diseases. However, such antibodies that comprise non-human portions elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the Ig derived protein. For example, repeated administration of antibodies comprising non-human portions can lead to serum sickness and/or anaphalaxis. In order to avoid these and other such problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and “humanization,” as well known in
the art. These approaches have produced antibodies having reduced immunogenicity, but with other less desirable properties.

Accordingly, there is a need to provide multiple sclerosis related human antibodies or specified portions or variants, nucleic acids, host cells, compositions, and methods of making and using thereof, that overcome one or more of these problems, as well as improvements over known human or humanized multiple sclerosis related protein antibodies or specified portions or variants thereof.

**SUMMARY OF THE INVENTION**

The present invention provides isolated multiple sclerosis related human Ig derived proteins (Ig derived proteins), including immunoglobulins, receptor fusion proteins, cleavage products and other specified portions and variants thereof, as well as multiple sclerosis related Ig derived protein compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and enabled herein, in combination with what is known in the art. Such multiple sclerosis related Ig derived proteins act as antagonists to multiple sclerosis related proteins and thus are useful for treated multiple sclerosis related pathologies. Multiple sclerosis related proteins include, but are not limited to IL-23 and IL-12, particularly p40 subunit of IL-23 and IL-12, as well as the p35 subunit of IL-12 or p19 subunit of IL-23.

The present invention also provides at least one isolated multiple sclerosis related Ig derived protein or specified portion or variant as described herein and/or as known in the art.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding specific multiple sclerosis related Ig derived proteins or specified portions or variants thereof, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said isolated multiple sclerosis related Ig derived protein nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such Ig derived protein nucleic acids, vectors and/or host cells.

At least one Ig derived protein or specified portion or variant of the invention binds at least one specified epitope specific to at least one multiple sclerosis related protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one Ig derived protein binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least one extracellular, soluble, hydrophilic, external or cytoplasmic portion of said protein. Non-limiting examples include at least one of 1-4, 7-11, 17-21, 27-41, 44-51, 53-59, 69-73, 76-81, 84-87, 91-95, 106-122, 126-130, 134-136, 149-170, 181-183, 211-219, 226-249, 253-270, 280-290, 293-302,
353-372, 391-401, 405-443, 451-470, 476-478, 486-497, and 490-503 of SEQ ID NO:1, where 1-197 of SEQ ID NO:1 includes the p35 subunit (197 amino acids) and 198-503 includes the human p40 subunit (306 amino acids), where binding to the p40 subunit is preferred.

The at least one Ig derived protein or specified portion or variant can optionally comprise at least one specified portion of at least one CDR (e.g., CDR1, CDR2 or CDR3 of the heavy or light chain variable region) and/or at least one framework region. The at least one Ig derived protein or specified portion or variant amino acid sequence can further optionally comprise at least one specified substitution, insertion or deletion.

The present invention also provides at least one composition comprising (a) an isolated multiple sclerosis related Ig derived protein or specified portion or variant encoding nucleic acid and/or Ig derived protein as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known methods. The composition can optionally further comprise at least one further compound, protein or composition.

The present invention also provides at least one method for expressing at least one multiple sclerosis related Ig derived protein or specified portion or variant in a host cell, comprising culturing a host cell as described herein and/or as known in the art under conditions wherein at least one multiple sclerosis related Ig derived protein or specified portion or variant is expressed in detectable and/or recoverable amounts.

The present invention further provides at least one multiple sclerosis related Ig derived protein, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of multiple sclerosis and related disorders, such as multiple sclerosis, type I or type II multiple sclerosis mellitus, including adult onset or juvenile, insulin dependent, non-insulin dependent, and the like, including the associated signs and symptoms, such as but not limited to, insulin resistance, hyperglycemia, hypoglycemia, pancreatitis, Sushing’s syndrome, acanthosis nigricans, lipoatrophic multiple sclerosis, retinopathy, nephropathy, polyneuropathy, mononeuropathy, autonomic neuropathy, ulcers, foot ulcers, joint problems, infections (e.g., fungal or bacterial), and the like, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art.

The present invention further provides at least one multiple sclerosis related Ig derived protein, specified portion or variant in a method or composition, when administered in a therapeutically effective amount, for modulation, for treating or reducing the symptoms of multiple sclerosis or multiple sclerosis related disease in a cell, tissue, organ, animal or patient and/or, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during a related disease or treatment condition, as known in the art and/or as described herein.
The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one multiple sclerosis related Ig derived protein or specified portion or variant, according to the present invention.

The present invention also provides at least one isolated multiple sclerosis related Ig derived protein, comprising at least one immunoglobulin complementarity determining region (CDR) or at least one ligand binding region (LBR) that specifically binds at least one multiple sclerosis related protein, wherein (a) said multiple sclerosis related Ig derived protein specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence, selected from the group consisting of the p40 subunit of a human interleukin-23 or interleukin-12 (298-503 of SEQ ID NO:1); interleukin-12 (SEQ ID NO:1, as 1-197 corresponding to the p35 subunit and 198-306 corresponding to the p40 subunit), such as but not limited to at least one of 198-215, 211-219, 226-249, 253-270, 280-290, 293-302, 353-372, 391-401, 405-443, 451-470, 476-478, 486-497 of SEQ ID NO:1, where 1-197 of SEQ ID NO:1 includes the p35 subunit (197 amino acids) and 198-503 includes the human p40 subunit (306 amino acids), where binding to the p40 subunit is preferred; said human Ig derived protein binds multiple sclerosis related with an affinity of at least 10⁻⁹ M, at least 10⁻¹¹ M, or at least 10⁻¹² M; said human Ig derived protein substantially neutralizes at least one activity of at least one multiple sclerosis related protein or hormone.

The invention also provides at least one isolated multiple sclerosis related human Ig derived protein encoding nucleic acid, comprising a nucleic acid that hybridizes under stringent conditions, or has at least 95% identity, to a nucleic acid encoding a multiple sclerosis related Ig derived protein. The invention further provides an isolated multiple sclerosis related human Ig derived protein, comprising an isolated human Ig derived protein encoded by such a nucleic acid. The invention further provides a multiple sclerosis related human Ig derived protein encoding nucleic acid composition, comprising such an isolated nucleic acid and a carrier or diluent. The invention further provides an Ig derived protein vector, comprising such a nucleic acid, wheein the vector optionally further comprises at least one promoter selected from the group consisting of a late or early SV40 promoter, a CMV promoter, an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, a human immunoglobulin promoter, or an EF-1 alpha promoter. Such a vector can optionally further comprise at least one selection gene or portion thereof selected from at least one of methotrexate (MTX), dihydrofolate reductase (DHFR), green fluorescent protein (GFP), neomycin (G418), or glutamine synthetase (GS). The invention further comprises a mammalian host cell comprising such an isolated nucleic acid, optionally wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.
The invention also provides at least one method for producing at least one multiple sclerosis related human Ig derived protein, comprising translating such a nucleic acid or an endogenous nucleic acid that hybridizes thereto under stringent conditions, under conditions in vitro, in vivo or in situ, such that the multiple sclerosis related human Ig derived protein is expressed in detectable or recoverable amounts.

The invention also provides at least one multiple sclerosis related human Ig derived protein composition, comprising at least one isolated multiple sclerosis related human Ig derived protein and a carrier or diluent, optionally further wherein said carrier or diluent is pharmaceutically acceptable, and/or further comprising at least one compound or protein selected from at least one of a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, a multiple sclerosis related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin, a filgrastim, a sargramostim, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylnethane, a cromolyn, an epinephrine or analog, dornase alpha, a cytokine, a cytokine antagonist.

The present invention also provides at least one method for treating a multiple sclerosis related condition in a cell, tissue, organ or animal, comprising contacting or administering a multiple sclerosis modulating effective amount of at least one multiple sclerosis related human Ig derived protein with, or to, said cell, tissue, organ or animal, optionally wherein said animal is a primate, optionally a monkey or a human. The method can further include where said multiple sclerosis related condition is at least one selected from multiple sclerosis, emphysema, asthma, chronic bronchitis or airflow obstruction, or optionally wherein said effective amount is 0.001-100 mg/kilogram of said cells, tissue, organ or animal. Such a method can further include wherein said contacting or said administering is by at least one mode selected from intravenous, intramuscular, bolus, intraperitoneal, subcutaneous, respiratory, inhalation, nasal, vaginal, rectal, buccal, sublingual, intranasal, subdermal, or transdermal. Such a method can further comprise administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising a therapeutically effective amount of at least one compound or protein selected from at least one of a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid,
an anabolic steroid, a multiple sclerosis related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin, a filgrastim, a sargramostim, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha, a cytokine, a cytokine antagonist.

The present invention also provides at least one medical device, comprising at least one multiple sclerosis related human Ig derived protein, wherein said device is suitable to contacting or administering said at least one multiple sclerosis related human Ig derived protein by at least one mode selected from intravenous, intramuscular, bolus, intraperitoneal, subcutaneous, respiratory, inhalation, nasal, vaginal, rectal, buccal, sublingual, intranasal, subdermal, or transdermal.

The present invention also provides at least one human immunoglobulin light chain multiple sclerosis related protein, comprising at least one portion of a variable region comprising at least one human Ig derived protein fragment of the invention.

The present invention also provides at least one human immunoglobulin heavy chain or portion thereof, comprising at least one portion of a variable region comprising at least one multiple sclerosis related human Ig derived protein fragment.

The invention also includes at least one human Ig derived protein, wherein said human Ig derived protein binds the same epitope or antigenic region as a multiple sclerosis related human Ig derived protein.

The invention also includes at least one formulation comprising at least one multiple sclerosis related human Ig derived protein, and at least one selected from sterile water, sterile buffered water, or at least one preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent, optionally wherein the concentration of multiple sclerosis related human Ig derived protein is about 0.1 mg/ml to about 100 mg/ml, further comprising at least one isotonicity agent or at least one physiologically acceptable buffer.

The invention also includes at least one formulation comprising at least one multiple sclerosis related human Ig derived protein according in lyophilized form in a first container, and an optional second container comprising at least one of sterile water, sterile buffered water, or at least one preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol,
benzyl alcohol, alkyloparaben, benzalkonium chloride, benzethonium chloride, sodium dehydroacetate
and thimerosal, or mixtures thereof in an aqueous diluent, optionally further wherein the concentration
of multiple sclerosis related human Ig derived protein is reconstituted to a concentration of about 0.1
mg/ml to about 500 mg/ml, further comprising an isotonicity agent, or further comprising a
physiologically acceptable buffer.

The invention further provides at least one method of treating at least one multiple sclerosis
related mediated condition, comprising administering to a patient in need thereof a formulation of the
invention.

The invention also provides at least one article of manufacture for human pharmaceutical use,
comprising packaging material and a container comprising a solution or a lyophilized form of at least
one multiple sclerosis related human Ig derived protein of the invention, optionally further wherein said
container is a glass or plastic container having a stopper for multi-use administration, optionally further
wherein said container is a blister pack, capable of being punctured and used in intravenous,
intramuscular, bolus, intraperitoneal, subcutaneous, respiratory, inhalation, nasal, vaginal, rectal,
sublingual, intranasal, subdermal, or transdermal administration; said container is a component
of an intravenous, intramuscular, bolus, intraperitoneal, subcutaneous, respiratory, inhalation, nasal,
vaginal, rectal, buccal, sublingual, intranasal, subdermal, or transdermal device or system; said
container is a component of an injector or pen-injector device or system for intravenous, intramuscular,
bolus, intraperitoneal, subcutaneous, respiratory, inhalation, nasal, vaginal, rectal, buccal, sublingual,
intranasal, subdermal, or transdermal.

The invention further provides at least one method for preparing a formulation of at least one
multiple sclerosis related human Ig derived protein of the invention, comprising admixing at least one
multiple sclerosis related human Ig derived protein in at least one buffer containing saline or a salt.

The invention also provides at least one method for producing at least one multiple sclerosis
related human Ig derived protein of the invention, comprising providing a host cell, transgenic animal,
transgenic plant or plant cell capable of expressing in recoverable amounts said human Ig derived
protein, optionally further wherein said host cell is a mammalian cell, a plant cell or a yeast cell; said
transgenic animal is a mammal; said transgenic mammal is selected from a goat, a cow, a sheep, a
horse, and a non-human primate.

The invention further provides at least one transgenic animal or plant expressing at least one
human Ig derived protein of the invention.

The invention further provides at least one multiple sclerosis related human Ig derived protein
produced by a method of the invention.

The invention further provides at least one method for treating at least one multiple sclerosis
related mediated disorder, comprising at least one of (a) administering an effective amount of a
composition or pharmaceutical composition comprising at least one multiple sclerosis related human Ig derived protein to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy; and further administering, before concurrently, and/or after said administering in (a) above, at least one selected from at least one of a multiple sclerosis therapeutic, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, a multiple sclerosis related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin, a filgrastim, a sargramostim, an immunizing agent, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimitabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, adonepezil, a tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, a dornase alpha, or a cytokine, a cytokine antagonist.

The present invention further provides any invention described herein. and is not limited to any particular description, embodiment or example provided herein.

DESCRIPTION OF THE INVENTION

Whereas the present scenario on multiple sclerosis treatment is decidedly grim, rapid advances in understanding its cellular and molecular pathophysiology give rise to hope that a new generation of drugs will emerge with the potential of slowing disease progression.

The present invention provides isolated, recombinant and/or synthetic multiple sclerosis related Ig derived proteins or specified portions or variants, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one multiple sclerosis related Ig derived protein. Such Ig derived proteins or specified portions or variants of the present invention comprise specific full length Ig derived protein sequences, domains, fragments and specified variants thereof, and methods of making and using said nucleic acids and Ig derived proteins or specified portions or variants, including therapeutic compositions, methods and devices.

As used herein, a “multiple sclerosis related Ig derived protein,” “multiple sclerosis related Ig derived protein portion,” or “multiple sclerosis related Ig derived protein fragment” and/or “multiple sclerosis related Ig derived protein variant” and the like decreases, blocks, inhibits, abrogates or interferes with multiple sclerosis related protein activity, binding or multiple sclerosis related protein receptor activity or binding in vitro, in situ and/or preferably in vivo.
For example, a suitable multiple sclerosis related Ig derived protein, specified portion or variant of the present invention can bind at least one multiple sclerosis related protein or receptor and includes anti-multiple sclerosis related Ig derived proteins, antigen-binding fragments thereof, and specified portions, variants or domains thereof that bind specifically to multiple sclerosis related. A suitable multiple sclerosis related Ig derived protein, specified portion, or variant can also decrease block, abrogate, interfere, prevent and/or inhibit multiple sclerosis related protein RNA, DNA or protein synthesis, multiple sclerosis related protein release, multiple sclerosis related protein or receptor signaling, membrane multiple sclerosis related protein cleavage, multiple sclerosis protein related activity, multiple sclerosis related protein production and/or synthesis, e.g., as described herein or as known in the art.

Anti-multiple sclerosis related Ig derived proteins (also termed multiple sclerosis related Ig derived proteins) useful in the methods and compositions of the present invention are characterized by high affinity binding to multiple sclerosis related proteins, and optionally and preferably having low toxicity. In particular, an Ig derived protein, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low immunogenicity, is useful in the present invention. The Ig derived proteins that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other suitable properties, may contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott et al., *Lancet* 344:1125-1127 (1994), each of the above references entirely incorporated herein by reference.

**Utility**

The isolated nucleic acids of the present invention can be used for production of at least one multiple sclerosis related Ig derived protein, fragment or specified variant thereof, which can be used to effect in an cell, tissue, organ or animal (including mammals and humans), to modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one multiple sclerosis related condition.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one anti-multiple sclerosis related Ig derived protein or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective
amount can comprise an amount of about 0.001 to 500 mg/kg per single or multiple administration, or
to achieve a serum concentration of 0.01-5000 μg/ml serum concentration per single or multiple
administration, or any effective range or value therein, as done and determined using known methods, as
described herein or known in the relevant arts.

Citations

All publications or patents cited herein are entirely incorporated herein by reference as they
show the state of the art at the time of the present invention and/or to provide description and
enablement of the present invention. Publications refer to any scientific or patent publications, or any
other information available in any media format, including all recorded, electronic or printed formats.
The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current
Lane, Ig derived proteins, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds.,
Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2003); Colligan et al., Current

Ig derived proteins of the Present Invention

The term "Ig derived protein "is intended to encompass Ig derived proteins, digestion
fragments, specified portions and variants thereof, including Ig derived protein mimetics or comprising
portions of Ig derived proteins that mimic the structure and/or function of an antibody or specified
fragment or portion thereof, including single chain Ig derived proteins and fragments thereof, and is
also is intended to encompass proteins that contain mimetics to therapeutic proteins, antibodies, and
digestion fragments, specified portions and variants thereof, wherein the protein comprises at least one
functional multiple sclerosis related protein ligand binding region (LBR) that optionally replaces at
least one complementarity determining region (CDR) of the antibody from which the Ig-derived protein,
portion or variant is derived. Such multiple sclerosis related IgG derived proteins, specified portions or
variants include those that mimic the structure and/or function of at least one multiple sclerosis related
protein antagonist, such as a multiple sclerosis related protein antibody or receptor or ligand protein, or
fragment or analog. Functional fragments include antigen-binding fragments that bind to human p40,
p35 or p19 proteins or fragments thereof. For example, Ig derived protein fragments capable of binding
to human p40, p35 or p19 proteins or fragments thereof, including, but not limited to Fab (e.g., by
papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and F(ab')2 (e.g., by pepsin
digestion), fabc (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by
pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology
techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).
Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Ig derived proteins can also be produced in a variety of truncated forms using Ig derived protein genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab')₂ heavy chain portion can be designed to include DNA sequences encoding the CH₁ domain and/or hinge region of the heavy chain. The various portions of Ig derived proteins can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, a nucleic acid encoding the variable and constant regions of a human Ig derived protein chain can be expressed to produce a contiguous protein. See, e.g., Colligan, Immunology, supra, sections 2.8 and 2.10, for fragmentation and Ladner et al., U.S. Patent No. 4,946,778 and Bird, R.E. et al., Science, 242: 423-426 (1988), regarding single chain Ig derived proteins, each of which publications are entirely incorporated herein by reference.

As used herein, the term "human Ig derived protein" refers to an Ig derived protein in which substantially every part of the protein (e.g., CDR, LBR, framework, C_L, C_H domains (e.g., C_H₁, C_H₂, C_H₃), hinge, (V_L, V_H)) is substantially non-immunogenic, with only minor sequence changes or variations. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans relative to non-modified human Ig derived proteins. Thus, a human Ig derived protein is distinct from a chimeric or humanized Ig. It is pointed out that a human Ig derived protein can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human Ig derived protein is a single chain Ig derived protein, it can comprise a linker peptide that is not found in native human Ig derived proteins. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin. multiple sclerosis related Ig derived proteins that comprise at least one multiple sclerosis related protein ligand or receptor thereof can be designed against an appropriate ligand, such as isolated and/or multiple sclerosis related protein, or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of such multiple sclerosis related Ig derived proteins are performed using known techniques to identify and characterize ligand binding regions or sequences of at least one multiple sclerosis related protein or portion thereof.

Human Ig derived proteins that are specific for the p40 subunit can be raised against an appropriate immunogenic antigen, such as isolated and/or multiple sclerosis related protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Preparation of immunogenic antigens, and monoclonal Ig derived protein production can be performed using any suitable technique. A variety of methods have been described (see e.g., Kohler et al., Nature, 256: 495-497 (1975) and
Eur. J. Immunol. 6: 511-519 (1976); Milstein et al., Nature 266: 550-552 (1977); Koprowski et al., U.S. Patent No. 4,172,124; Harlow, E. and D. Lane, 1988, Ig derived proteins: A Laboratory Manual, (Cold Spring Harbor Laboratory: Cold Spring Harbor, NY); Current Protocols In Molecular Biology, Vol. 2 (e.g., Supplement 27, Summer '94), Ausubel, F.M. et al., Eds., (John Wiley & Sons: New York, NY), Chapter 11, (1991-2003), each of which is entirely incorporated herein by reference. Generally, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art, see, e.g., www.atcc.org, www.lifetech.com., and the like, each of which is entirely incorporated herein by reference) with Ig derived protein producing cells, such as, but not limited to, isolated or cloned spleen cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, lhnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, each entirely incorporated herein by reference.

Ig derived protein producing cells can be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an Ig derived protein, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce Ig derived proteins with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BiolInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, CA; Ixsys. See, e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; US 08/350260(5/12/94); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC);
WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); WO96/13583, WO97/08320 (MorphoSys); WO95/16027 (BiolInvent); WO88/06630; WO90/3809 (Dyax); US 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or proteins - US 5723323, 5763192, 5814476, 5817483, 5824514, 5976862, WO 86/05803, EP 590 689 (Ixsys, now

Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immunol. 41:901-907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95-118 (1996); Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and applications) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein.


Methods for humanizing non-human Ig derived proteins can also be used and are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature 321:522 (1986);

Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988), each of which is entirely incorporated herein by reference), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" Ig derived proteins are chimeric Ig derived proteins (Cabilly et al., supra), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized Ig derived proteins are typically human Ig derived proteins in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent Ig derived proteins.

The choice of human variable domains, both light and heavy, to be used in making the humanized Ig derived proteins can be used to reduce antigenicity. According to the so-called "best-fit"
5 method, the sequence of the variable domain of a rodent antibody is screened against the entire library
of known human variable-domain sequences. The human sequence which is closest to that of the rodent
is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol.
151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), each of which is entirely
incorporated herein by reference). Another method uses a particular framework derived from the
consensus sequence of all human Ig derived proteins of a particular subgroup of light or heavy chains.
The same framework can be used for several different humanized Ig derived proteins (Carter et al.,
is entirely incorporated herein by reference).

Ig derived proteins can also optionally be humanized with retention of high affinity for the
antigen and other favorable biological properties. To achieve this goal, according to a preferred method,
humanized Ig derived proteins are prepared by a process of analysis of the parental sequences and
various conceptual humanized products using three-dimensional models of the parental and humanized
sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to
those skilled in the art. Computer programs are available which illustrate and display probable three-
dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of
these displays permits analysis of the likely role of the residues in the functioning of the candidate
immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate
immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the
consensus and import sequences so that the desired antibody characteristic, such as increased affinity
for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially
involved in influencing antigen binding.

Human monoclonal Ig derived proteins can be made by the hybridoma method. Human
myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal Ig
derived proteins have been described, for example, by Kozbor, J. Immunol. 133:3001 (1984); Brodeur
et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc.,
New York, 1987); and Boerner et al., J. Immunol. 147:86 (1991), each of which is entirely incorporated
herein by reference.

Alternatively, phage display technology and as presented above can be used to produce human
Ig derived proteins and antibody fragments in vitro, from immunoglobulin variable (V) domain gene
repertoires from unimmunized donors. According to one none limiting example of this technique,
antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a
filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the
surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of
the phage genome, selections based on the functional properties of the antibody also result in selection
of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson et al., Current Opinion in Structural Biology 3:564 (1993), each of which is entirely incorporated herein by reference. Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624 (1991) isolated a diverse array of anti-oxazolone Ig derived proteins from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and Ig derived proteins to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581 (1991), or Griffith et al., EMBO J. 12:725 (1993), each of which is entirely incorporated herein by reference.

In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technol. 10:779 (1992)). In this method, the affinity of "primary" human Ig derived proteins obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of Ig derived proteins and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., Nucl. Acids Res. 21:2265 (1993). Gene shuffling can also be used to derive human Ig derived proteins from rodent Ig derived proteins, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent Ig derived proteins obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection with antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT WO 93/06213, published 1 April 1993). Unlike traditional humanization of rodent Ig derived proteins by CDR grafting, this technique provides completely human Ig derived proteins, which have no framework or CDR residues of rodent origin.

Bispecific Ig derived proteins can also be used that are monoclonal, preferably human or humanized, Ig derived proteins that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one multiple sclerosis related protein, the other one is for any other antigen. For example, bispecific Ig derived proteins specifically binding a multiple sclerosis related protein and at least one neurotrophic factor, or two different types of multiple
sclerosis related polypeptides are within the scope of the present invention.

Methods for making bispecific Ig derived proteins are known in the art. Traditionally, the recombinant production of bispecific Ig derived proteins is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published 13 May 1993, and in Traunecker et al., EMBO J. 10:3655 (1991), entirely incorporated herein by reference.

According to a different and more preferred approach, antibody-variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, the second heavy chain constant region (C.sub.H 2), and the third heavy chain constant region (C.sub.H 3). It is preferred to have the first heavy-chain constant region (C.sub.H 1), containing the site necessary for light-chain binding, present in at least one of the fusions. DNA sequences encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the production of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific Ig derived proteins are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. For further details of generating bispecific Ig derived proteins, see, for example, Suresh et al., Methods in Enzymology 121:210 (1986).

Heteroconjugate Ig derived proteins are also within the scope of the present invention. Heteroconjugate Ig derived proteins are composed of two covalently joined Ig derived proteins. Such Ig derived proteins have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/00373; and EP 03089). Heteroconjugate Ig derived proteins can be made using any convenient cross-linking methods.
Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

In a preferred embodiment, at least one anti-multiple sclerosis related Ig derived protein or specified portion or variant of the present invention is produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells. Immortalized multiple sclerosis related producing cells can be produced using suitable methods, for example, fusion of a human Ig derived protein-producing cell and a heteromyeloma or immortalization of an activated human B cell via infection with Epstein Barr virus (Niedbala et al., Hybridoma, 17(3):299-304 (1998); Zanella et al., J Immunol Methods, 156(2):205-215 (1992); Gustafsson et al., Hum Ig derived proteins Hybridomas, 2(1):26-32 (1991)). Preferably, the human anti-human p40, p35 or p19 proteins or fragments or specified portions or variants is generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human Ig derived proteins, as described herein and/or as known in the art. Cells that produce a human anti-human p40, p35 or p19 Ig derived protein can be isolated from such animals and immortalized using suitable methods, such as the methods described herein.


The term "functionally rearranged," as used herein refers to a segment of DNA from an immunoglobulin locus that has undergone V(D)J recombination, thereby producing an immunoglobulin gene that encodes an immunoglobulin chain (e.g., heavy chain, light chain), or any portion thereof. A functionally rearranged immunoglobulin gene can be directly or indirectly identified using suitable
methods, such as, for example, nucleotide sequencing, hybridization (e.g., Southern blotting, Northern blotting) using probes that can anneal to coding joints between gene segments or enzymatic amplification of immunoglobulin genes (e.g., polymerase chain reaction) with primers that can anneal to coding joints between gene segments. Whether a cell produces an Ig derived protein comprising a particular variable region or a variable region comprising a particular sequence (e.g., at least one CDR sequence) can also be determined using suitable methods. In one example, mRNA can be isolated from an Ig derived protein-producing cell (e.g., a hybridoma or recombinant cell or other suitable source) and used to produce cDNA encoding the Ig derived protein or specified portion or variant thereof. The cDNA can be cloned and sequenced or can be amplified (e.g., by polymerase chain reaction or other known and suitable methods) using a first primer that anneals specifically to a portion of the variable region of interest (e.g., CDR, coding joint) and a second primer that anneals specifically to non-variable region sequences (e.g., C_H1, V_H).

Screening Ig derived protein or specified portion or variants for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure.

Ig derived protein screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA), and Cambridge Ig derived protein Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4704692, 4939666, 4946778, 5260203, 5455030, 5518889, 5534621, 5656730, 5763733, 5767260, 5856456, assigned to Enzon; 5223409, 5403484, 5571698, 5837500, assigned to Dyax, 5427908, 5580717, assigned to Affymax; 5885793, assigned to Cambridge Ig derived protein Technologies; 5750373, assigned to Genentech, 5618920, 5595898, 5576195, 5698435, 5693493, 5698417, assigned to Xoma, Colligan, supra; Ausubel, supra; or Sambrook, supra, each of the above patents and publications entirely incorporated herein by reference.

Ig derived proteins, specified portions and variants of the present invention can also be prepared using at least one multiple sclerosis related Ig derived protein or specified portion or variant encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep,
and the like, that produce such Ig derived proteins or specified portions or variants in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, US patent nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Ig derived proteins, specified portions and variants of the present invention can additionally be prepared using at least one multiple sclerosis related Ig derived protein or specified portion or variant encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such Ig derived proteins, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., Curr. Top. Microbiol. Immunol. 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. Ig derived proteins have also been produced in large amounts from transgenic plant seeds including Ig derived protein fragments, such as single chain Ig derived proteins (scFv’s), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101-109 (1998) and reference cited therein. Thus, Ig derived proteins, specified portions and variants of the present invention can also be produced using transgenic plants, according to known methods. See also, e.g., Fischer et al., Biotechnol. Appl. Biochem. 30:99-108 (Oct., 1999), Ma et al., Trends Biotechnol. 13:522-7 (1995); Ma et al., Plant Physiol. 109:341-6 (1995); Whitlam et al., Biochem. Soc. Trans. 22:940-944 (1994); and references cited therein. See, also generally for plant expression of Ig derived proteins, but not limited to, Each of the above references is entirely incorporated herein by reference.

The Ig derived proteins of the invention can bind human p40, p35 or p19 proteins or fragments with a wide range of affinities (K_D). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human p40, p35 or p19 proteins or fragments with high affinity. For example, a human mAb can bind human p40, p35 or p19 proteins or fragments with a K_D equal to or less than about 10^-9 M or, more preferably, with a K_D equal to or less than about 0.1-9.9 (or any range or value therein) X 10^-10 M, 10^-11, 10^-12, 10^-13 or any range or value therein.

The affinity or avidity of an Ig derived protein for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Ig derived protein-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular Ig derived protein-antigen interaction can vary
if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity
and other antigen-binding parameters (e.g., $K_D$, $K_B$, $K_A$) are preferably made with standardized solutions
of Ig derived protein and antigen, and a standardized buffer, such as the buffer described herein.

**Nucleic Acid Molecules**

Using the information provided herein, such as the nucleotide sequences encoding at least 90-
100% of the contiguous amino acids of at least one of multiple sclerosis related Ig derived protein of
the present invention, specified fragments, variants or consensus sequences thereof, or a deposited
vector comprising at least one of these sequences, a nucleic acid molecule of the present invention
encoding at least one multiple sclerosis related Ig derived protein or specified portion or variant can be
obtained using methods described herein or as known in the art.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA,
hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and
_genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA
can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of
at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it
can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention can include nucleic acid molecules
comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to,
at least one specified portion of at least one CDR, as CDR1, CDR2 and/or CDR3 of at least one heavy
chain or light chain, respectively; nucleic acid molecules comprising the coding sequence for a multiple
sclerosis related Ig derived protein or specified portion or variant; and nucleic acid molecules which
comprise a nucleotide sequence substantially different from those described above but which, due to the
degeneracy of the genetic code, still encode at least one multiple sclerosis related Ig derived protein as
described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus,
it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code
for specific multiple sclerosis related Ig derived protein or specified portion or variants of the present
invention. See, e.g., Ausubel, et al., _supra_, and such nucleic acid variants are included in the present
invention.

In another aspect, the invention provides isolated nucleic acid molecules encoding a(n) multiple
sclerosis related Ig derived protein or specified portion or variant having an amino acid sequence as
encoded by the nucleic acid contained in the plasmid deposited as designated clone names
______________________________ and ATCC Deposit Nos.
______________________________, respectively, deposited on
______________________________.
As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding a multiple sclerosis related Ig derived protein or specified portion or variant can include, but are not limited to, those encoding the amino acid sequence of an Ig derived protein fragment, by itself; the coding sequence for the entire Ig derived protein or a portion thereof; the coding sequence for an Ig derived protein, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an Ig derived protein or specified portion or variant can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused Ig derived protein or specified portion or variant comprising an Ig derived protein fragment or portion.

**Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein**

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide encoding a multiple sclerosis related Ig derived protein of the present invention. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of an Ig derived protein or specified portion or variant encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding an Ig derived protein or specified portion or variant of the present invention. See, e.g., Ausubel, supra; Colligan, supra, each entirely incorporated herein by reference.
Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Recombinant Methods for Constructing Nucleic Acids

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Nucleic Acid Screening and Isolation Methods

A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of
complementarity will optimally be 100%, or 90-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Patent No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Patent No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). The T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

**Synthetic Methods for Constructing Nucleic Acids**

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

**Recombinant Expression Cassettes**
The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an Ig derived protein or specified portion or variant of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

A polynucleotide of the present invention can be expressed in either sense or anti-sense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable characteristics.

Another method of suppression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to block the transcription of target genes.


Vectors And Host Cells

The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one multiple sclerosis related Ig derived protein or specified portion or variant by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.
The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat.Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017, ampicillin, neomycin (G418), mycohenolic acid, or glutamine synthetase (GS, US Pat.Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

At least one Ig derived protein or specified portion or variant of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of an Ig derived protein or specified portion or variant to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to an Ig derived protein or specified portion or variant of the present invention to facilitate purification. Such regions can be removed prior to final preparation of an Ig derived protein or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.
Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an Ig derived protein or specified portion or variant of the present invention. Such methods are well known in the art, e.g., as described in US patent Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

Illustrative of cell cultures useful for the production of the Ig derived proteins, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (US Pat.Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (US Pat.No. 5,266,491), at least one human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

**Purification of an Ig derived protein or Specified Portion or Variant Thereof**

A multiple sclerosis related Ig derived protein or specified portion or variant can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to,
protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2003), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

Ig derived proteins or specified portions or variants of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the Ig derived protein or specified portion or variant of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely incorporated herein by reference.

20 MULTIPLE SCLEROSIS RELATED Ig DERIVED PROTEINS, FRAGMENTS AND/OR VARIANTS

The isolated Ig derived proteins of the present invention comprise an Ig derived protein or specified portion or variant encoded by any one of the polynucleotides of the present invention as discussed more fully herein, or any isolated or prepared Ig derived protein or specified portion or variant thereof.

Preferably, the human Ig derived protein or antigen-binding fragment binds human p40, p35 or p19 proteins or fragments and, thereby substantially neutralizes the biological activity of the protein. An Ig derived protein, or specified portion or variant thereof, that partially or preferably substantially neutralizes at least one biological activity of at least one multiple sclerosis related protein or fragment can bind the protein or fragment and thereby inhibit activitys mediated through the binding of multiple sclerosis related to the multiple sclerosis related receptor or through other multiple sclerosis related-dependent or mediated mechanisms. As used herein, the term "neutralizing Ig derived protein" refers to an Ig derived protein that can inhibit human p40, p35 or p19 protein or fragment related-dependent activity by about 20-120%, preferably by at least about 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The capacity of anti-human p40, p35 or p19 Ig derived protein or specified portion or variant to inhibit human p40, p35 or p19 related-dependent activity is preferably assessed by at least one suitable multiple sclerosis related Ig derived protein or protein assay, as described herein and/or as known in the art. A human Ig derived protein or specified portion or variant of the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.) or isotype and can comprise a kappa
or lambda light chain. In one embodiment, the human Ig derived protein or specified portion or variant comprises an IgG heavy chain or defined fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. Ig derived proteins of this type can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one human light chain (e.g., IgG, IgA and IgM (e.g., γ1, γ2, γ3, γ4) transgenes as described herein and/or as known in the art. In another embodiment, the anti-human p40, p35 or p19 Ig derived protein or specified portion or variant thereof comprises an IgG1 heavy chain and a IgG1 light chain.

At least one Ig derived protein or specified portion or variant of the invention binds at least one specified epitope specific to at least one multiple sclerosis related protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one Ig derived protein binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least one extracellular, soluble, hydrophilic, external or cytoplasmic portion of said protein. As non-limiting examples, (a) a multiple sclerosis related Ig derived protein or specified portion or variant specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence, selected from the group consisting of at least one subunit of human IL-12 or IL-23. The at least one specified epitope can comprise any combination of at least one amino acid of the p40 subunit of a human interleukin-23 or interleukin-12 (298-503 of SEQ ID NO:1); interleukin-12 (SEQ ID NO:1, as 1-197 corresponding to the p35 subunit and 198-306 corresponding to the p40 subunit), such as but not limited to at least one of 198-215, 211-219, 226-249, 253-270, 280-290, 293-302, 353-372, 391-401, 405-443, 451-470, 476-478, 486-497 of SEQ ID NO:1, where 1-197 of SEQ ID NO:1 includes the p35 subunit (197 amino acids) and 198-503 includes the human p40 subunit (306 amino acids).

Generally, the human Ig derived protein or antigen-binding fragment of the present invention will comprise an antigen-binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one heavy chain variable region and at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the Ig derived protein or antigen-binding portion or variant can comprise at least one of the heavy chain CDR3, and/or a light chain CDR3. In a particular embodiment, the Ig derived protein or antigen-binding fragment can have an antigen-binding region that comprises at least a portion of at least one heavy chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3. In another particular embodiment, the Ig derived protein or antigen-binding portion or variant can have an antigen-binding region that comprises at least a portion of at least one light chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3. Such Ig derived proteins can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the Ig derived protein using conventional techniques, by preparing and expressing a (i.e., one or
more) nucleic acid molecule that encodes the Ig derived protein using conventional techniques of recombinant DNA technology or by using any other suitable method.

The anti-human p40, p35 or p19 Ig derived protein can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. For example, in a preferred embodiment, the anti-human p40, p35 or p19 Ig derived protein comprises at least one of at least one heavy chain variable region and/or at least one light chain variable region. Human Ig derived proteins that bind to human p40, p35 or p19 proteins or fragments and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such as phage display (Katsube, Y., et al., Int J Mol. Med., 1(5):863-868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light chain locus that can undergo functional rearrangement, can be immunized with human p40, p35 or p19 proteins or fragments thereof to elicit the production of Ig derived proteins. If desired, the Ig derived protein producing cells can be isolated and hybridomas or other immortalized Ig derived protein-producing cells can be prepared as described herein and/or as known in the art.

Alternatively, the Ig derived protein, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

The invention also relates to Ig derived proteins, antigen-binding fragments, immunoglobulin chains and CDRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such Ig derived proteins or antigen-binding fragments and Ig derived proteins comprising such chains or CDRs can bind human p40, p35 or p19 proteins or fragments with high affinity (e.g., Kp less than or equal to about 10^9 M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g., charge, structure, polarity, hydrophobicity/hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

**Amino Acid Codes**

The amino acids that make up multiple sclerosis related Ig derived proteins or specified portions or variants of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three
nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The

<table>
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<th>SINGLE LETTER CODE</th>
<th>THREE LETTER CODE</th>
<th>NAME</th>
<th>THREE NUCLEOTIDE CODON(S)</th>
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<tr>
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<td>Ala</td>
<td>Alanine</td>
<td>GCA, GCC, GCG, GCU</td>
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<tr>
<td>C</td>
<td>Cys</td>
<td>Cysteine</td>
<td>UGC, UGU</td>
</tr>
<tr>
<td>D</td>
<td>Asp</td>
<td>Aspartic acid</td>
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<td>Glu</td>
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<td>F</td>
<td>Phe</td>
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<td>G</td>
<td>Gly</td>
<td>Glycine</td>
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A multiple sclerosis related Ig derived protein or specified portion or variant of the present
invention can include one or more amino acid substitutions, deletions or additions, either from natural
mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on
many factors, including those described above. Generally speaking, the number of amino acid
substitutions, insertions or deletions for any given multiple sclerosis related polypeptide will not be
more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1-30 or any
range or value therein, as specified herein.

Amino acids in a multiple sclerosis related Ig derived protein or specified portion or variant of
the present invention that are essential for function can be identified by methods known in the art, such
as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15;
Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single
alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for
biological activity, such as, but not limited to at least one multiple sclerosis related neutralizing activity. Sites that are critical for Ig derived protein or specified portion or variant binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

A(n) multiple sclerosis related Ig derived protein or specified portion or variant can further optionally comprise a polypeptide of at least one of 1-50% of the contiguous amino acids of at least one of SEQ ID NOS: 7, 8, 9, 10, 11, 12.

The Ig derived proteins or specified portions or variants of the present invention, or specified variants thereof, can comprise any number of contiguous amino acid residues from an Ig derived protein or specified portion or variant of the present invention, wherein that number is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in a multiple sclerosis related Ig derived protein or specified portion or variant. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes at least one biologically active Ig derived protein or specified portion or variant of the present invention. Biologically active Ig derived proteins or specified portions or variants have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-100% of that of the native (non-synthetic), endogenous or related and known Ig derived protein or specified portion or variant. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to human Ig derived proteins and antigen-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce an Ig derived protein or antigen-binding fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.
The modified Ig derived proteins and antigen-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the Ig derived protein or specified portion or variant. Each organic moiety that is bonded to an Ig derived protein or antigen-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term “fatty acid” encompasses mono-carboxylic acids and di-carboxylic acids. A “hydrophilic polymeric group,” as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, an Ig derived protein modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying Ig derived proteins of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrolidone. Preferably, the hydrophilic polymer that modifies the Ig derived protein of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG5000 and PEG20,000, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used.

The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying Ig derived proteins of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying Ig derived proteins of the invention include, for example, n-dodecanoate (C12, laurate), n-tetradecanoate (C14, myristate), n-octadecanoate (C18, stearate), n-eicosanoate (C20, arachidate), n-docosanoate (C22, behenate), n-triacontanoate (C30), n-tetracontanoate (C40), cis-Δ9-octadecanoate (C18, olate), all cis-Δ5,8,11,14-eicosatetraenoate (C20, arachidonate), octanediolic acid, tetradecanediolic acid, octadecanediolic acid, docosanediolic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human Ig derived proteins and antigen-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A “modifying agent” as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty
acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidy esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorus group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C1-C12 group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, -(CH₂)₃-,
-NH(CH₂)₆-NH-, -CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH₂-O-CH-NN-. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylendiamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, et al., WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified Ig derived proteins of the invention can be produced by reacting a human Ig derived protein or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the Ig derived protein in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human Ig derived proteins or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an Ig derived protein or antigen-binding fragment. The reduced Ig derived protein or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified Ig derived protein of the invention. Modified human Ig derived proteins and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an Ig derived protein or specified portion or variant of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch et al., *Bioconjugate Chem.*, 3:147-153 (1992); Werlen et al., *Bioconjugate Chem.*, 5:411-417 (1994); Kumaran et al., *Protein Sci.* 6(10):2233-2241 (1997); Itoh et al., *Bioorg. Chem.*, ...
MULTIPLE SCLEROSIS RELATED Ig DERIVED PROTEIN OR SPECIFIED PORTION OR VARIANT COMPOSITIONS

The present invention also provides at least one multiple sclerosis related Ig derived protein or specified portion or variant composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more multiple sclerosis related Ig derived proteins or specified portions or variants thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the multiple sclerosis related Ig derived protein amino acid sequence, or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.

Multiple sclerosis related Ig derived protein or specified portion or variant compositions of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., Remington’s Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the multiple sclerosis related composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/Ig derived protein or specified portion or variant components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.
Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrins, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

Multiple sclerosis related Ig derived protein compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, the multiple sclerosis related Ig derived protein or specified portion or variant compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as “TWEEN 20” and “TWEEN 80”), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the multiple sclerosis related compositions according to the invention are known in the art, e.g., as listed in “Remington: The Science & Practice of Pharmacy”, 19th ed., Williams & Williams, (1995), and in the “Physician’s Desk Reference”, 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

**Formulations**

As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one multiple sclerosis related Ig derived protein or specified portion or variant in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxycetanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or
mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in
the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005,
0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5,
1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8,
3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no
preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3: 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9,
1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05,
0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkyloparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005,
0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising
packaging material and at least one vial comprising a solution of at least one multiple sclerosis related
Ig derived protein or specified portion or variant with the prescribed buffers and/or preservatives,
optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that
such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72
hours or greater. The invention further comprises an article of manufacture, comprising packaging
material, a first vial comprising lyophilized at least one multiple sclerosis related Ig derived protein or
specified portion or variant, and a second vial comprising an aqueous diluent of prescribed buffer or
preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute
the at least one multiple sclerosis related Ig derived protein or specified portion or variant in the
aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one multiple sclerosis related Ig derived protein or specified portion or variant used
in accordance with the present invention can be produced by recombinant means, including from
mammalian cell or transgenic preparations, or can be purified from other biological sources, as
described herein or as known in the art.

The range of at least one multiple sclerosis related Ig derived protein or specified portion or
variant in the product of the present invention includes amounts yielding upon reconstitution, if in a
wet/dry system, concentrations from about 1.0 μg/ml to about 1000 mg/ml, although lower and higher
concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution
formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump
methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable
preservative. Preferred preservatives include those selected from the group consisting of phenol, m-
cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkyloparaben (methyl, ethyl, propyl, butyl and
the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or
mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient
to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyls, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one multiple sclerosis related Ig derived protein or specified portion or variant and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one multiple sclerosis related Ig derived protein or specified portion or variant and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one multiple sclerosis related Ig derived protein or specified portion or variant in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one multiple sclerosis related Ig derived protein or specified
portion or variant that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biologically activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

The solutions of at least one multiple sclerosis related Ig derived protein or specified portion or variant in the invention can be prepared by a process that comprises mixing at least one Ig derived protein or specified portion or variant in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one Ig derived protein or specified portion or variant in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that may be optimized for the concentration and means of administration used.

The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one multiple sclerosis related Ig derived protein or specified portion or variant that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one multiple sclerosis related Ig derived protein or specified portion or variant that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one Ig derived protein or specified portion or variant solution can be retrieved.
one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as BD Pens, BD Autojector®, Humaject®, NovoPen®, B-D® Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotropinorm Pen®, Humatro Pen®, Recol-Pen®, Roferon Pen®, Biojector®, Iject®, J-tip Needle-Free Injector®, Intraject®, Medi-Ject®, e.g., as made or developed by Becton Dickensen (Franklin Lakes, NJ, www.bectondickenson.com), Disetronic (Burgdorf, Switzerland, www.disetronic.com; Bioject, Portland, Oregon (www.bioject.com); National Medical Products , Weston Medical (Peterborough, UK, www.weston-medical.com), Medi-Ject Corp (Minneapolis, MN, www.medject.com). Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen®.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one multiple sclerosis related Ig derived protein or specified portion or variant in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one multiple sclerosis related Ig derived protein or specified portion or variant and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one Ig derived protein or specified portion or variant and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one Ig derived protein or specified portion or variant in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one multiple sclerosis related Ig derived protein or specified portion or variant that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial
requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

At least one multiple sclerosis related Ig derived protein or specified portion or variant in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

Therapeutic Applications

The present invention also provides a method for modulating or treating multiple sclerosis related conditions, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of multiple sclerosis, type I or type II multiple sclerosis mellitus, including adult onset or juvenile, insulin dependent, non-insulin dependent, and the like, including the associated signs and symptoms, such as but not limited to, insulin resistance, hyperglycemia, hypoglycemia, pancreatitis, Sushing’s syndrome, acanthosis nigricans, lipoatrophic multiple sclerosis, retinopathy, nephropathy, polyneuropathy, mononeuropathy, autonomic neuropathy, ulcers, foot ulcers, joint problems, infections (e.g., fungal or bacterial), and the like. Such a method can optionally comprise administering an effective amount of at least one composition or pharmaceutical composition comprising at least one multiple sclerosis related Ig derived protein or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at least one multiple sclerosis associated immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of type I or type II multiple sclerosis mellitus, including adult onset or juvenile, insulin dependent, non-insulin dependent, and the like, including the associated signs and symptoms, such as but not limited to, insulin resistance, hyperglycemia, hypoglycemia, pancreatitis, Sushing’s syndrome, acanthosis nigricans, lipoatrophic multiple sclerosis, retinopathy, nephropathy, polyneuropathy, mononeuropathy, autonomic neuropathy, ulcers, foot ulcers, joint problems, infections (e.g., fungal or bacterial), and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, NJ (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2001), each entirely incorporated by reference.

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one multiple sclerosis related Ig derived protein or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one
multiple sclerosis related Ig derived protein, specified portion or variant thereof, further comprises
administering, before concurrently, and/or after, at least one selected from at least one multiple
sclerosis therapeutic (including but not limited to, beta-interferon 1a and beta-interferon 1b (e.g.,
Avonex™, Rebif™, Betaseon™), glutiramer acetate (e.g., Copaxone), cyclophosphamide, azathioprine,
glucocorticosteroids, methotrexate, Paclitaxel, 2-chlorodeoxyadenosine, mitoxantrone, IL-10, TGBb,
CD4, CD52, antegren, CD11, CD18, TNFalpha, IL-1, IL-2, and/or CD4 antibody or antibody receptor
fusion, interferon alpha, immunoglobulin, Lismide (Requinimax™), insulin-like growth factor-1 (IGF-
1), elprolid, pirfenidone, oral myelin, or compounds that act on one or more of at least one of:
autoimmune suppression of myelin destruction, immune regulation, activation, proliferation, migration
and/or suppressor cell function of T-cells, inhibition of T cell receptor/peptide/MHC-II interaction,
Induction of T cell anergy, deletion of autoreactive T cells, reduction of trafficking across blood brain
barrier, alteration of balance of pro-inflammatory (Th1) and immunomodulatory (Th2) cytokines,
inhibition of matrix metalloprotease inhibitors, neuroprotection, reduction of gliosis, promotion of re-
myelination), TNF antagonist (e.g., but not limited to a TNF Ig derived protein or fragment, a soluble
TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an
antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an
analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g.,
aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a
fluorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an
antipsoriatic, a corticosteroid, an anabolic steroid, a multiple sclerosis related agent, a mineral, a
nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antiarrheal, an antitussive, an
antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim
(e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin,
an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone
replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an
antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, animanic agent, an
antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma
medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an
epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable
dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition,
Appleton and Lange, Stamford, CT (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia
2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are
entirely incorporated herein by reference.

TNF antagonists suitable for compositions, combination therapy, co-administration, devices
and/or methods of the present invention (further comprising at least one antibody, specified portion and
variant thereof, of the present invention), include, but are not limited to, anti-TNF Ig derived proteins, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g., pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g., captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor Ig derived protein," "TNFα Ig derived protein," "TNFα Ig derived protein," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNFα activity in vitro, in situ and/or preferably in vivo. For example, a suitable TNF human Ig derived protein of the present invention can bind TNFα and includes anti-TNF Ig derived proteins, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNFα. A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

Chimeric Ig derived protein cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNFα IgG1 Ig derived protein, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic Ig derived protein effector function, increases the circulating serum half-life and decreases the immunogenicity of the Ig derived protein. The avidity and epitope specificity of the chimeric Ig derived protein cA2 is derived from the variable region of the murine Ig derived protein A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine Ig derived protein A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNFα in a dose dependent manner. From binding assays of chimeric Ig derived protein cA2 and recombinant human TNFα, the affinity constant of chimeric Ig derived protein cA2 was calculated to be 1.04x10^6 M^-1. Preferred methods for determining monoclonal Ig derived protein specificity and affinity by competitive inhibition can be found in Harlow, et al., Ig derived proteins: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-2003); Kozbor et al., Immunol. Today, 4:72-79 (1983); Ausubel et al., eds. Current Protocols in

In a particular embodiment, murine monoclonal Ig derived protein A2 is produced by a cell line designated c134A. Chimeric Ig derived protein cA2 is produced by a cell line designated c168A.


TNF Receptor Molecules

Preferred TNF receptor molecules useful in the present invention are those that bind TNFα with high affinity (see, e.g., Feldmann et al., International Publication No. WO 92/07076 (published April 30, 1992); Schall et al., Cell 61:361-370 (1990); and Loetscher et al., Cell 61:351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran et al., Eur. J. Biochem. 223:831-840 (1994)), are also useful in the present invention. Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNFα inhibitory binding proteins (Engelmann, H. et al., J. Biol. Chem. 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, may contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric
molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer et al., Eur. J. Immunol. 21:2883-2886 (1991); Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Peppel et al., J. Exp. Med. 174:1483-1489 (1991); Kolls et al., Proc. Natl. Acad. Sci. USA 91:215-219 (1994); Butler et al., Cytokine 6(6):616-623 (1994); Baker et al., Eur. J. Immunol. 24:2040-2048 (1994); Beutler et al., U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon et al., U.S. Patent No. 5,116,964; Capon et al., U.S. Patent No. 5,225,538; and Capon et al., Nature 337:525-531 (1989), which references are entirely incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNFα with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNFα with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience, New York (1987-2003).

Cytokines include any known cytokine. See, e.g., CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any Ig derived protein, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

**Therapeutic Treatments.** Any method of the present invention can comprise a method for treating a multiple sclerosis related mediated disorder, comprising administering an effective amount of
a composition or pharmaceutical composition comprising at least one multiple sclerosis related Ig
derived protein or specified portion or variant to a cell, tissue, organ, animal or patient in need of such
modulation, treatment or therapy.

Typically, treatment of pathologic conditions is effected by administering an effective amount or
dosage of at least one multiple sclerosis related Ig related protein composition that total, on average, a
range from at least about 0.01 to 500 milligrams of at least one multiple sclerosis related Ig derived protein
or specified portion or variant /kilogram of patient per dose, and preferably from at least about 0.1 to 100
milligrams Ig derived protein or specified portion or variant /kilogram of patient per single or multiple
administration, depending upon the specific activity of contained in the composition. Alternatively, the
effective serum concentration can comprise 0.1-5000 μg/ml serum concentration per single or multiple
administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the
particular disease state, specific activity of the composition being administered, and the particular patient
undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to
provide for repeated administration, i.e., repeated individual administrations of a particular monitored or
metered dose, where the individual administrations are repeated until the desired daily dose or effect is
achieved.

Preferred doses can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8,
9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37,
38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67,
68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96,
97, 98, 99 and/or 100 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum
concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0,
6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 20, 12.5, 12.9, 13.0, 13.5,
13.9, 14.0, 14.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11,
11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14, 14.5, 15, 15.5, 15.9, 16, 16.5, 17, 17.5, 17.9, 18, 18.5,
18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75,
80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000,
4500, and/or 5000 μg/ml serum concentration per single or multiple administration, or any range, value or
fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the
pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age,
health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment,
frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to
100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams
per kilogram per administration or in sustained release form is effective to obtain desired results.
As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one Ig derived protein or specified portion or variant of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

For parenteral administration, the Ig derived protein or specified portion or variant can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Alternative Administration

Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one multiple sclerosis related Ig derived protein or specified portion or variant according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

multiple sclerosis related Ig derived proteins of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

Parenteral Formulations and Administration
Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

Alternative Delivery

The invention further relates to the administration of at least one multiple sclerosis related Ig derived protein or specified portion or variant by parenteral, subcutaneous, intramuscular, intravenous, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. Protein, Ig derived protein or specified portion or variant compositions can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as creams and suppositories; for buccal, or sublingual administration particularly in the form of tablets or capsules; or intranasally particularly in the form of powders, nasal drops or aerosols or certain agents; or transdermally particularly in the form of a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

Pulmonary/Nasal Administration

For pulmonary administration, preferably at least one multiple sclerosis related Ig derived protein or specified portion or variant composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one multiple sclerosis
related Ig derived protein or specified portion or variant can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of Ig derived protein or specified portion or variants are also known in the art. All such devices can use of formulations suitable for the administration for the dispensing of Ig derived protein or specified portion or variant in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx™ Aradigm, the Ultravent® nebulizer (Mallinckrodt), and the Acorn II™ nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one multiple sclerosis related Ig derived protein or specified portion or variant is delivered by a dry powder inhaler or a sprayer. There are several desirable features of an inhalation device for administering at least one Ig derived protein or specified portion or variant of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10 μm, preferably about 1-5 μm, for good respirability.

Administration of multiple sclerosis related Ig derived protein or specified portion or variant Compositions as a Spray

A spray including multiple sclerosis related Ig derived protein or specified portion or variant composition protein can be produced by forcing a suspension or solution of at least one multiple sclerosis related Ig derived protein or specified portion or variant through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one multiple sclerosis related Ig derived protein or specified portion or variant composition protein delivered by a sprayer
have a particle size less than about 10 µm, preferably in the range of about 1 µm to about 5 µm, and most preferably about 2 µm to about 3 µm.

Formulations of at least one multiple sclerosis related Ig derived protein or specified portion or variant composition protein suitable for use with a sprayer typically include Ig derived protein or specified portion or variant composition protein in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one multiple sclerosis related Ig derived protein or specified portion or variant composition protein per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, .1, .2, , .3, .4, .5, .6, .7, .8, .9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the Ig derived protein or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating Ig derived protein or specified portion or variant composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating Ig derived protein or specified portion or variant composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The Ig derived protein or specified portion or variant composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the Ig derived protein or specified portion or variant composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as multiple sclerosis related Ig derived proteins, or specified portions or variants, can also be included in the formulation.

**Administration of multiple sclerosis related Ig derived protein or specified portion or variant compositions by a Nebulizer**

Ig derived protein or specified portion or variant composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of Ig derived protein or specified portion or variant composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-
frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of Ig derived protein or specified portion or variant composition protein either directly or through a coupling fluid, creating an aerosol including the Ig derived protein or specified portion or variant composition protein. Advantageously, particles of Ig derived protein or specified portion or variant composition protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one multiple sclerosis related Ig derived protein or specified portion or variant suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one multiple sclerosis related Ig derived protein or specified portion or variant protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one multiple sclerosis related Ig derived protein or specified portion or variant composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one multiple sclerosis related Ig derived protein or specified portion or variant composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one multiple sclerosis related Ig derived protein or specified portion or variant include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one multiple sclerosis related Ig derived protein or specified portion or variant formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one multiple sclerosis related Ig derived protein or specified portion or variant caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as Ig derived protein or specified portion or variant protein can also be included in the formulation.

Administration of multiple sclerosis related Ig derived protein or specified portion or variant compositions By A Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, at least one multiple sclerosis related Ig derived protein or specified portion or variant, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of Ig derived protein or specified portion or variant
composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation; or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one multiple sclerosis related Ig derived protein or specified portion or variant for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one multiple sclerosis related Ig derived protein or specified portion or variant as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluoralkane-134a), HFA-227 (hydrofluoralkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one multiple sclerosis related Ig derived protein or specified portion or variant as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one multiple sclerosis related Ig derived protein or specified portion or variant compositions via devices not described herein.

Oral Formulations and Administration

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasyrol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution
preparations allowable for medical use. These preparations may contain inactive diluting agents
ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems
for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of
mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673).
Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5871,753 are
used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration

For absorption through mucosal surfaces, compositions and methods of administering at least
one multiple sclerosis related Ig derived protein or specified portion or variant include an emulsion
comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and
an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving
mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for
application of the emulsions of the present invention can include corneal, conjunctival, buccal,
sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration.
Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for
example, polyalkylene glycols, vaseline, cocoa butter, and the like. Formulations for intranasal
administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily
solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate,
magnesium stearate, pregelatinated starch, and the like (U.S. Pat. Nos. 5,849,695).

Transdermal Formulations and Administration

For transdermal administration, the at least one multiple sclerosis related Ig derived protein or
specified portion or variant is encapsulated in a delivery device such as a liposome or polymeric
nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles
unless otherwise stated). A number of suitable devices are known, including microparticles made of
synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and
copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such
as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and
combinations thereof (U.S. Pat. Nos. 5,814,599).

Prolonged Administration and Formulations

It can be sometimes desirable to deliver the compounds of the present invention to the subject
over prolonged periods of time, for example, for periods of one week to one year from a single
administration. Various slow release, depot or implant dosage forms can be utilized. For example, a
dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low
degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as
phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid,
polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-
ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt.
Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactide/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1: Cloning and Expression of multiple sclerosis related immunoglobulin protein in Mammalian Cells

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the Ig derived protein or specified portion or variant coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human
Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded Ig derived protein or specified portion or variant. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of Ig derived protein or specified portion or variants.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp7l8, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of multiple sclerosis related Ig derived protein or specified portion or variant. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified
gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV1.

Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the multiple sclerosis related in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete multiple sclerosis related Ig derived protein or specified portion or variant is used, corresponding to HC and LC variable regions of a multiple sclerosis related Ig derived protein of the present invention, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct (e.g., as provided in vector p1351).

The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 μg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with
10, 25, or 50 ng/ml of methotrexate plus 1 μg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

The completely human anti-multiple sclerosis related protein Ig derived proteins are further characterized. Several of generated Ig derived proteins are expected to have affinity constants between 1x10^9 and 9x10^12. Such high affinities of these fully human monoclonal Ig derived proteins make them suitable for therapeutic applications in multiple sclerosis related protein-dependent diseases, pathologies or related conditions.

EXAMPLE 2: Use of IL-12 antibodies of the present invention in EAE model of Multiple Sclerosis

Abstract

IL-12 is a pro-inflammatory cytokine that is produced by antigen presenting cells (APC) and promotes differentiation of Th1 effector cells. IL-12 is present in the developing lesions of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) models. Interference of IL-12 pathways was shown to effectively prevent EAE in rodents. The EAE model in the common marmoset approximates the human disease MS. This holds true for the clinical presentation, as well as for the neuropathological and radiological (MRI) aspects of the lesions that can be found in the brain and spinal cord. These aspects, together with the close genetic and immunological proximity with humans makes the marmoset EAE model an excellent test system for biotechnologically engineered therapeutics, with exclusive reactivity in primates.

In this study we report on the beneficial effect of p40 huMab (also termed 12B75 or C340), a human monoclonal Ab (mAb) against human IL-12p40, in the myelin-induced EAE model in common marmosets. Treatment was initiated well after immunization (day 14) and the mAb remained active throughout the treatment period of 72 days. We demonstrate that p40 huMab treatment has a protective effect on clinical expression as well as on the MRI-detectable and neuropathological changes normally
seen in EAE affected individuals, stressing the clinical potential of this therapeutic agent for the
treatment of MS.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). The
pathological hallmark of MS is the CNS white matter lesion, a focal area of infiltrated mononuclear
cells with a variable degree of demyelination, axonal loss and gliosis. Although susceptibility to MS is
thought to be a multifactorial trait, it is generally assumed that disease progression is driven by
autoimmune reactions directed to antigens within the CNS white matter (1-2). Recently, detailed
pathological analysis revealed that in MS four fundamentally different patterns can be recognized (3).
Pattern I and pattern II lesion pathology are modeled in viral- and autoimmune-based animal models of
encephalomyelitis in susceptible rodent strains (4-6) as well as in non-human primates (7).

IL-12 is the predominant cytokine for triggering Th1-mediated (autoimmune) responses and is induced
when CD4⁺ T-cells and antigen presenting cells (APC) interact (8-10) and abrogated when CD40-
CD154 ligation is disturbed (11-13). Evidence is accumulating that IL-12 plays a pivotal role in the
induction of autoimmune responses that are critical in the initiation of experimental autoimmune
encephalomyelitis (EAE), during lesion formation, and for progression of the disease (11-22).
Therapies directed at neutralization of IL-12 or prevention of production by abrogation of CD40-
CD154 conjugation have proven to be effective in rodent (20, 21, 23-28) as well as in marmoset EAE
(29, 30), while excess of IL-12 reverses these effects and enhances EAE severity (15-17, 22, 28, 31).
Furthermore, local expression of IL-12 within the CNS of rodents (14, 32, 33) and common marmosets
(34) associated with active EAE has been established.

Although the situation is less clear in MS, local expression of IL-12 occurs within the CNS of affected
individuals (35-37), and levels of IL-12 in CSF and plasma are increased during active disease (38-40).
Moreover, it has been argued that the beneficial effect of IFN-β on MS may be exerted via suppression
of IL-12 production (41-43). Finally, lower base-line levels of IL-12p35 and p40 mRNA seem
predictive for clinical responsiveness to IFN-β treatment (44,45).

In its radiological and neuropathological presentation, the chronic progressive EAE model in the
common marmoset approximates the most prevalent lesion type, being pattern II, in MS patients (7, 46,
47). These aspects, added to the MS-like clinical expression of the disease (7, 48) and the close
immunological similarity with humans (49-51), makes the model an excellent test system for preclinical
evaluation of new MS therapies. The model is particularly important for testing biotechnologically
engineered therapeutics, which by their species-specificity can not be evaluated in rodent EAE models.
The present study describes the beneficial effects of p40 huMab, a fully human IgG molecule directed against human IL-12p40 on the clinical features of EAE. Moreover, we show a reduction of the lesion load, as detected with in vivo and post-mortem magnetic resonance imaging (MRI) as well as by neuropathological examination.

Materials and Methods

Animals

Ten non-related healthy common marmosets (*Callithrix jacchus*) were purchased from the experimental stock of the Biomedical Primate Research Centre (Rijswijk, The Netherlands). Before the monkeys entered the experiment a full physical, hematological and biochemical check-up was performed. Individual data of the monkeys, which were identified with an implanted transponder, are summarized in table 1.

Before immunization the monkeys were random
supplemented with rice, raisins, peanuts, marshmallows, biscuits and fresh fruit. Drinking water was provided ad libitum. According to the Dutch law on animal experimentation, the experimental procedures of this study have been reviewed and approved by the Institute's Animal Care and User Committee.

Disease induction and clinical read-out

EAE was induced by a single immunization with 300 μl human myelin in water (10 mg/ml) emulsified in CFA (Difco Laboratories, Detroit, MI) under ketamine anesthesia 6 mg/kg (AST Farma, Oudewater, The Netherlands) as described previously (52). Bordetella pertussis administration was omitted for reasons discussed elsewhere (52).

Twice daily, clinical signs of EAE were scored blind by a trained observer using a previously described semi-quantitative scale (52): 0, no clinical signs; 0.5, apathy, loss of appetite and altered walking pattern without ataxia; 1, lethargy and/or anorexia; 2, ataxia, sensory loss/blindness; 2.5, hemi- or paraparesis; 3, hemi- or paraplegia; 4, quadriplegia; 5, spontaneous death attributable to EAE. Body weights were determined once weekly as a surrogate disease marker. Monkeys were sacrificed for ethical reasons once an EAE score 3.0 had been reached, or at day 86 after immunization, being the end of the study period.

Reactivity, dosing regimen, plasma levels, biodistribution and immunogenicity of p40 huMab

The test substance was produced by clone C379B and purified using standard techniques. p40 huMab is a fully human IgG,kappa mAb specific for the p40 subunit of human IL-12. The effectiveness of p40 huMab in neutralizing marmoset IL-12 was tested using lipopolysaccharide (LPS) stimulated cells. Briefly, adherent cells from both common marmosets and human peripheral blood mononuclear cells (PBMC) were treated for 24 h with LPS (1 μg/ml). Cell free supernatant was collected and titrated onto 4 day PHA-stimulated human T-cell blasts. After 24 h, IFN-α levels were determined using standard ELISA techniques. An EC₅₀ stimulation level was determined for each preparation and used at this concentration for titrating p40 huMab to determine the IC₅₀ of neutralization.

After induction of EAE the animals were treated between day 14 and day 86 after immunization (a.i.). Five animals received weekly intravenous (i.v.) injections of 10 mg/kg p40 huMab in saline into the vena saphena under ketamin anesthesia. Control animals (n=5) received weekly i.v. injections with sterile saline (0.9% NaCl, 1 ml/kg) as placebo treatment.
At three days after each dosing, serum p40 huMab levels were determined using an ELISA. Briefly, recombinant human IL-12 (10 μg/ml) was coated on 96 wells plates (Costar Corning, New York, NY). Plasma samples were incubated and after washing, p40 huMab was detected by mouse-anti-human Fc Ab, grown from cell line HP6017 (ATCC, Manasas, VA). The Ab was purified via protein A, coupled with sulfo-N-hydroxysuccinimide LC biotin (Pierce, Rockford IL), and developed using horse radish peroxidase conjugated streptavidin (SA-HRP; Jackson ImmunoResearch Laboratories, West Grove, MA). Concentrations were calculated using a standard curve prepared with p40 huMab. Anti-12B75 Ab levels were determined by a sandwich ELISA using p40 huMab binding to the plate and detected with biotinylated p40 huMab (12B75-biotin) and SA-HRP as described above.

Magnetic Resonance Imaging

High-resolution MRI experiments were performed on a 4.7 T horizontal bore Varian NMR spectrometer (Varian, Palo Alto, CA), equipped with a high-performance gradient insert (11 cm inner diameter, maximum gradient strength 220 mT/m). For in vivo MRI, animals were anesthetized with 30 mg/kg ketamin in combination with 1 mg/kg valium (Diazepam; Kombivet BV, Etten-Leur, The Netherlands). MRI data sets were collected for T1-weighted (T1-w) and T2-w images. A bird cage volume coil (diameter 9 cm) was used for radio frequency transmission and signal reception. For all sequences the field of view was 4x4 cm (matrix 128x128; zero filled to 256x256; in plane resolution 312x312 μm). Post-contrast T1-w images were made after i.v. injection of 0.3 mmol/kg gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA; Magnevist, Schering AG, Berlin Germany). Post-mortem high contrast T2-w images were made of formalin-fixed brains. A solenoidal-coil (4 windings; diameter 35 mm) was used for radio frequency transmission and signal reception (field of view 3x3 cm; matrix 128x128; zero-filling 256x256; in plane resolution 240x240 μm). The in vivo and post-mortem MRI scores were calculated as proposed by Jordan and coworkers (52). The data sets were analyzed on an Apple Macintosh I-MAC G3 (Apple Computer, Cupertino, CA) using the public domain National Institute of Health Program (NIH Image 1.52).

Neuropathological examination

After formalin fixation, parts of the brain, spinal cord and peripheral nerves were embedded in paraffin and processed as described previously (29). In brief, the cerebrum and cerebellum were divided in 7 or 8 coronal cut parts and the spinal cord was dissected transversely. The extent of inflammation, demyelination and axonal pathology was evaluated on 3-5 μm tissue sections stained with hematoxylin/eosin (HE) to visualize infiltrated cells, Kläver Barrera Luxol Fast Blue (LFB) combined with Periodic Acid Schiff (PAS) for myelin and myelin degradation products, and with Bielschowsky
silver impregnation for axons. The degree of inflammation was expressed as an index being calculated as the average number of inflamed blood vessels per spinal cord section (n = 10 to 15 sections). Furthermore, the amount of demyelination was quantified on 10 to 15 spinal cord fields using a monomorphic grid.

In situ detection of i.v. injected p40 huMab.

To determine whether p40 huMab is able to cross the blood-brain-barrier and gain access to the lesions in the CNS, 1 mg of 12B75-biotin was i.v. injected 1 h before sacrifice into placebo-treated monkeys (Mi-031 and Mi-038) with EAE score of 3.0. For detection of 12B75-biotin frozen tissue sections of brain and spleen were incubated with SA-HRP (Jackson) for 1 h at room temperature (RT), followed by tyramide signal amplification (TSA; NEN Life Science Products, Boston, MA). HRP-activity was revealed by incubation for 10 min at RT with 3-amino-9-ethyl-carbazole (AEC; Sigma, Zwijndrecht, The Netherlands), resulting in a red precipitate. To assess whether the p40 huMab mAb colocalizes with astrocytes, tissue sections were double stained using a polyclonal donkey-anti-human Ab directed towards glial fibrillary acidic protein (GFAP).

Cytokine profiles in brain and spinal cord

Immunohistochemistry was performed as previously described (34, 54) with minor modifications. Frozen sections were cut, thaw-mounted on glass slides, and kept overnight at RT in an humidified atmosphere. After air-drying for 1 h, slides were fixed in fresh acetone containing 0.02% H₂O₂ (v/v), air-dried for 10 min, washed with PBS, and incubated with primary Abs overnight at 4°C. Incubations with secondary and tertiary reagents were performed for 1 h at RT. Between incubation steps slides were washed twice with PBS. HRP activity was revealed by incubation for 10 min at RT with AEC, leading to a bright red precipitate. Detection of primary unlabeled mouse Abs were followed by an incubation with rabbit-anti-mouse-Ig-HRP (Dako, Glostrup, Denmark), or in case of a three-step staining with rabbit-anti-mouse-Ig-biotin (Dako) and HRP labeled avidin-biotin-complex (ABC/HRP; Dako). Primary Abs derived from rabbits were detected with biotin labeled donkey-anti-rabbit-Ig (Amersham, Little Chalfont, UK) as a second step.

For detection of IFN-γ and TNF-α the MD-2 and 61E71 mAb were used, respectively (U-Cytech, Utrecht, the Netherlands). MAb against IL-4 and IL-6 were derived from Genzyme (Cambridge, MA). C8.6, a mouse Ab for the detection of IL-12p40, was from Pharmingen (San Diego, CA). IL-10 was visualized using the B-S10 mAb (Instruchemie, Hilversum, The Netherlands). The Ab against IL-18, M318, was derived from R&D Systems (Abingdon, United Kingdom). 2D9, an mouse mAb for the detection of matrix metallo protease 9 (MMP-9), was a kind gift from G. Opdenakker (REGA Institute,
Leuven, Belgium). Both CD3 and reactive nitrogen and oxygen species (i-NOS) Abs were polyclonal and derived from Dako and Calbiochem (San Diego, CA), respectively. Finally, cells expressing CD40 were detected using a mouse-anti-human CD40 mAb (5D12; Tanox Pharma Inc., HO, TX).

**T- and B-cell functions**

At necropsy, PBMC were isolated from heparinized blood using Lymphocyte Separation Medium (LSM®; ICN Biomedical Inc., Aurora, OH). Cell suspensions were prepared and cultured from aseptically removed lymph node (LNC) and spleen (SC) and cultured as described previously in the presence of recombinant human myelin oligodendrocyte glycoprotein (rhMOG; 10 µg/ml) or human myelin basic protein (hMBP; 25 µg/ml) to determine primary responses.

Serum was isolated from blood collected without coagulant, drawn via a needle puncture into the vena saphena. Sera were collected from paired animals at the pre-immunization stage, when one of the monkeys in a pair displayed EAE score 2.0, and again at the time of necropsy. Standard ELISA assays were used to determine IgM and IgG Ab levels directed to MBP and MOG as described (29).

**Statistics**

The $\chi^2$-test was used to determine statistical significant treatment related effects on the survival time; MannWhitney-$U$ test for anti-MOG and anti-MBP Ab levels and for MRI-scores. In all cases, P<0.05 was considered statistically significant.

**Results**

**Effect of p40 huMab on clinical EAE**

The time points for each animal when clinical signs were first observed, when a clinical score of 2.0 was reached, and when an animal was sacrificed for severe disease (score 3.0) are given in table I above. The percentages of maximal weight loss during the experiment are depicted in the same table. The results show a beneficial effect of p40 huMab treatment on the clinical expression of EAE. Four placebo-treated monkeys developed severe progressive EAE and one had a period of mild EAE during the observation period of 86 days. In the p40 huMab-treated group only monkey, Mi-019, developed clinical signs of EAE (P < 0.001 vs. placebo treatment; $\chi^2$-test). Notably, the period between disease onset and the maximally accepted clinical signs in this monkey was considerably longer than in the EAE-affected placebo-treated monkeys (see table I).
Cross-reactivity, serum levels and anti-Ab responses of p40 huMab

p40 huMab was able to neutralize the IFN-γ inducing properties conditioned medium derived from LPS stimulated marmoset adherent cells with comparable IC₅₀ as human conditioned media. At 3 days after each administration of p40 huMab, plasma levels generally maintained a concentration of 50 to 75 µg/ml. This is approximately a 50-fold excess of the IC₅₀ determined in vitro. In animal Mi-026 an unexplained disappearance and subsequent reappearance of p40 huMab serum levels was observed. No Ab responses directed against p40 huMab were detectable and no alterations in hematological and biochemical parameters were observed during the study period.

MRI

The *a priori* condition set before the experiment was to subject a monkey pair to MRI analysis once one of the animals of that pair has reached EAE score 2.0 (ataxia), irrespective of the clinical condition of the other monkey. Because of the acute onset of the disease in Mi-032 and Mi-043, both animals had to be euthanized for ethical reasons before an *in vivo* MRI could be performed. A characteristic feature of myelin-induced EAE is a strong periventricular inflammatory reaction visible as hyperintense regions of interest (ROI) in T2-w brain MR-images, and as hypointense ROI on T1-w images. In all tested placebo-treated animals comparable T2-w hyperintensities were observed, including the animal that only developed a mild clinical EAE (Mi-038). In sharp contrast, no such abnormalities were detectable in p40 huMab-treated animals. A dramatic effect of p40 huMab treatment on MRI-detectable changes in the brains of monkey pair 4 was determined. In the placebo-treated monkey Mi-031 large hyperintense ROI were found at the ventral horn of both lateral ventricles at EAE score 2.0. These alterations were completely absent in the clinically normal p40 huMab-treated monkey Mi-024, recorded at the same day (B). Although not significant, post-mortem MRI scores of p40 huMab-treated animals tended to be lower when compared to the scores of control animals (*P* = 0.08; Mann-Whitney *U*-test).

Neuropathology

All control animals showed cellular infiltrates in the brain and spinal cord. Three placebo-treated animals showed extensive demyelination which was mainly localized in the spinal cord, while in the animal with the mild EAE (Mi-038) the CNS pathology was mainly localized in the brain (see table 1). Although inflammation and demyelination of the CNS was detected in 2 mAb-treated individuals, 3 animals (Mi-003; Mi-023; Mi-026) were completely devoid of CNS pathology in the spinal cord and the brain (*P* = 0.03 for inflammation and *P* = 0.07 for demyelination; Mann-Whitney *U*-test). Placebo-
treated animal Mi-032 displayed specific demyelination of the CNS in the spinal cord, while no such pathological changes were observed in the p40 huMab-treated animal of this pair, Mi-026.

**Immunohistochemistry**

Injected 12B75-biotin into placebo-treated animals was detected as granular staining around the ventricles as well as in the cellular infiltrates within the brain. Staining was found located on astrocytes surrounding these infiltrates. The number of IL-12p40 positive astrocytes decreased further away from these areas. IL-12p40 was found in some cells within brain infiltrates, and on many astrocytes.

The amount as well as the size of the infiltrates found in the brains of placebo-treated animals were higher when compared to p40 huMab-treated individuals. Staining for IL-12p40 did not show any difference in both groups. Cellular infiltrates in the brain of asymptomatic antibody-treated animals showed decreased positive staining for MMP-9, IFN-γ, IL-4 and TNF-α as compared to placebo-treated animals. Antibody-treated animals that did show CNS pathology resembled EAE-affected animals in the placebo-treated group. While T cells were present in the cellular infiltrates of EAE affected monkeys, CD3⁺ cells were undetectable in the brains of 3 out of 4 mAb-treated animals that remained clinically healthy. No clear differences were observed in the number of IL-6, IL-10, IL-18, and i-NOS positive cells between the two experimental groups.

**Anti-myelin T-cell reactivity**

To investigate whether the suppressive effect of p40 huMab on the disease expression is due to suppression of anti-myelin T-cell activity we determined the proliferative responses of PBMC, SC and LNC against rhMOG and hMBP. In animals developing EAE relatively early after disease induction, significant proliferative responses to MOG were detectable in both SC and LNC. In contrast, in all animals sacrificed at the end-point of the study (both placebo and p40 huMab-treated animals), T-cell reactivity was mainly restricted to the spleen, while LNC responses had waned. While in all monkeys the T-cell response towards MBP was low, high responses were found against MOG (MOG vs. MBP: P < 0.01; Mann-Whitney U-test).

**Anti-myelin B-cell reactivity**

Anti-MOG Abs have been reported to have a critical role in this model mediating demyelination (46, 55-58). We therefore determined the effect of p40 huMab treatment on anti-MBP and anti-MOG Ab levels in the sera of placebo and p40 huMab-treated animals using ELISA. Anti-MOG and anti-MBP IgM Abs were hardly detectable at any of the chosen time points in the circulation of monkeys from
both groups. High anti-MOG and anti-MBP IgG serum levels were found in placebo-treated animals sampled at EAE-score 2.0, while lower IgG Ab levels were found in sera from p40 huMab-treated animals (P = 0.05; Mann Whitney U-test). At necropsy, significantly lower MBP-specific IgG levels were present in the sera of clinically healthy p40 huMab-treated animals (P = 0.03).

Discussion

In this article we report on the beneficial effects of neutralizing IL-12 p40 p40 huMab in the common marmoset model of EAE. To our knowledge this report is the first documented evidence that human Abs produced by transgenic mice are effective in a non-human primate autoimmune disease model. Despite extensive engineering of murine Ig's thereby eliminating the vast majority of the original mouse variant, the host's immune response to the foreign protein molecule is still the limiting factor for a long-standing effect of mAb therapy (59, 60). A possible solution to this dilemma may be the recent generation of transgenic mice that produce functionally intact human Abs after immunization (61). However, the lack of cross reactivity observed in rodents renders non-human primates as the only ethical alternative for safety and efficacy testing of such compounds. p40 huMab (also termed 12B75 and C340) is a human IgG1 mAb specific for the p40 subunit of human IL-12 with comparable bioneutralization of marmoset and human IL-12 in vitro. The fact that remarkably stable p40 huMab serum levels were measured throughout the treatment period of 72 days indicates that the mAb is not or minimally immunogenic in vivo, despite the use of CFA for disease induction. In contrast, treatment with humanized CD40 already results in substantial neutralizing Ab activity within two weeks after initiation (29).

Since all marmosets are susceptible to EAE after immunization with human myelin (7) and the fact that T- and B-cell responses could be detected in all p40 huMab-treated animals, it is highly unlikely that these protected monkeys were potential non-responders. In this study all placebo-treated monkeys developed clinical signs of EAE. In contrast, 4 out of 5 p40 huMab-treated animals remained clinically asymptomatic. Notably, the disease progression in the one p40 huMab-treated animal that developed EAE was substantially suppressed when compared to placebo-treated monkeys. An important feature of this study is that MRI-detectable changes in the CNS white matter were diminished in p40 huMab-treated monkeys. A clear visible difference was that the periventricular inflammatory reaction observed in all tested placebo-treated monkeys tested was absent in p40 huMab-treated monkeys. Moreover, 3 out of 5 p40 huMab-treated monkeys remained completely devoid of CNS inflammation and demyelination and infiltration of CD3+ T-cells into the brain and spinal cord was also prevented.
Although microglia are an important source for IL-12 (10, 63), we demonstrate that also astrocytes stain positive for intracellular IL-12p40. IL-12 is expressed at sites where T-cells and APCs interact (8-10). In the common marmoset EAE model such sites are the secondary lymphoid organs and the developing lesions within the CNS white matter (34, 54, 62). Injected 12B75-biotin was detected at both locations, confirming a previous finding that during active disease the blood-brain-barrier is permeable for large molecules like IgG (30). This observation indicates that p40 huMab can act locally in the CNS at places where infiltrating T-cells interact with resident APC or infiltrating macrophages. Interestingly, recent *in vitro* studies revealed that p40 huMab also neutralizes human IL-23 (D. Peritt, unpublished data), a cytokine that seems to play a pivotal role in the activation of memory CD4⁺CD45RB(low) T-cells (64) and may play an important role in autoimmune disease such as MS.

The different patterns of T-cell reactivity seen in PBMC, LNC and SC to MBP and MOG seems to be associated with disease development. The recent observation of Targoni and colleagues (65) that T-cell autoreactivity wanes in time from draining lymph nodes and the circulation during EAE development, but stays present in the spleen is in line with our observations. However, since all p40 huMab-treated monkeys displayed a similar reaction pattern as the placebo-treated monkey sacrificed at the same time point, we assume that p40 huMab treatment only partially suppresses the development of autoreactive T-cells yet does not interfere with the cell circulation kinetics. Possibly, p40 huMab treatment deviates myelin-reactive T-cells into the Th2 direction as was observed in mice (21), but this hypothesis has to be investigated in more detail.

Anti-myelin Abs are thought to be a critical factor in CNS demyelination in the marmoset model (46, 55-58). It is therefore of interest that circulating Ab levels to MOG and MBP at the time of the first clinical signs of neurological dysfunction (score 2 or ataxia) were significantly reduced in p40 huMab-treated animals when compared to placebo-treated animals. At time of necropsy no differences in anti-MOG Ab levels were determined between p40 huMab- and placebo-treated animals. As the presence of anti-MOG antibodies is a necessary condition for EAE development, this finding points at the possibility that the beneficial effect of p40 huMab is partly due to modulation of the auto-antibody reaction. This finding supports similar observations with anti-CD40 antibody treatment (29). Although the time points of sacrifice between the two groups differ substantially, this observation seems in contrast with the data published by McFarland and colleagues who showed that the appearance of anti-MOG Abs in the circulation was strongly linked with the onset of clinical signs of EAE induced with MP4 (57).

T-cell infiltrates as well as IFN-γ and IL-4 production were not detectable in the majority of mAb-treated animals. Moreover, inflammatory mediators like TNF-α and MMP-9 were not expressed in
these brains while all tested immune mediators were expressed in the brain infiltrates of all placebo-treated animals. In contrast, intracellular IL-12p40 expression inside the CNS white matter did not differ between placebo- and p40 huMab-treated animals. Although the mAb could also interfere APC derived IL-12 activation of T cells in the secondary lymphoid organs, it is tempting to speculate that p40 huMab prevents EAE development in common marmosets by interfering in the IL-12 mediated activation of T-cells at those sites of the CNS were they normally infiltrate the CNS white matter.

Finally, the relatively late stage that treatment was initiated (day 14 after immunization) excludes p40 huMab interference with EAE onset, but rather with the post-immunization processes that lead to clinical EAE. These facts taken together with the low immunogenicity of p40 huMab warrant further testing of this monoclonal antibody as a potential new therapy for MS.

References


30. Laman, J. D., H. P. M. Brok, submitted for publication.


Example 3: The role of IL-12p40 in marmoset EAE

Keywords: EAE/MS, immunotherapy, Th1/Th2, autoimmunity, neuroimmunology
Abstract

The experimental autoimmune encephalomyelitis (EAE) model in the common marmoset approximates the human disease multiple sclerosis (MS) with regard to its clinical presentation, as well as neuropathological and radiological aspects of the lesions in the brain and spinal cord. IL-12 is a pro-inflammatory cytokine that is produced by APC and promotes differentiation of Th1 effector cells. IL-12 is believed to be produced in the developing lesions of patients with MS as well as in EAE affected animals. Previously it was shown that interference in IL-12 pathways effectively prevents EAE in rodents. In this study we report that in vivo neutralization of IL-12p40 reduced EAE pathogenesis in the myelin-induced EAE model in common marmosets. Treatment was initiated well after immunization (day 14) and the mAb remained active throughout the treatment period of 72 days. During this period anti-Ab responses could not be detected. We demonstrate that anti-IL-12p40 treatment has a protective effect on the neurological dysfunction as well as on neuropathological changes normally observed in the brain and spinal cord of EAE affected individuals.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the CNS. The pathological hallmark of MS is the CNS white-matter lesion, a focal area of infiltrated mononuclear cells with a variable degree of demyelination, axonal loss and gliosis. Although susceptibility to MS is thought to be a multifactorial trait, it is generally accepted that disease progression is driven by autoimmune reactions directed against antigens of the CNS white matter (1, 2). A broad pathological analysis revealed that in MS at least four fundamentally different neuropathological patterns can be discerned (3). Pattern I and pattern II lesion pathology are modeled in the current virus- and autoimmune-based animal models of encephalomyelitis that have been established in susceptible rodent strains (4-6) or non-human primates (7).

IL-12 is the predominant cytokine for triggering Th1-mediated (autoimmune) responses and is induced when CD4+ T-cells and APC interact (8-10) and abrogated when CD40-CD154 ligation is disturbed (11-13). Evidence is accumulating that IL-12 plays a pivotal role in the induction of the critical autoimmune responses involved in the initiation of experimental autoimmune encephalomyelitis (EAE), lesion formation, and the progression of the disease (11-22). Therapies directed at the neutralization of IL-12 or prevention of production by abrogation of CD40-CD154 interaction have proven to be effective in rodent (20, 21, 23-28) as well as in marmoset models of EAE (29), while excess of IL-12 reverses these effects and enhances EAE severity (15-17, 22, 30). Furthermore, local expression of IL-12 within the CNS of rodents (14, 31, 32) and common marmosets (33) during active EAE has been demonstrated.
Although the situation is less clear in MS, IL-12 is locally expressed within the CNS of affected individuals (34-36), and levels of IL-12 in CSF and plasma are increased during active disease (37-39). Moreover, it has been argued that the beneficial effect of IFN-β on MS is exerted via suppression of IL-12 production (40-42). Finally, lower base-line levels of IL-12p35 and p40 mRNA seem to predict clinical responsiveness to IFN-β treatment (43, 44).

In its radiological and neuropathological presentation, the chronic progressive EAE model in the common marmoset approximates the most prevalent lesion type, being pattern II, in MS patients (7, 45, 46). These aspects, added to the MS-like clinical expression of the disease (7, 47) and the close immunological similarity with humans (48-50), make the model an excellent test system to study the biology of chronic MS. The present study demonstrates that a monoclonal antibody directed against IL-12p40 significantly reduces the lesion load of EAE, as detected with magnetic resonance imaging (MRI) and neuropathological examination.

Materials and Methods

Animals

Ten non-related healthy common marmosets (*Callithrix jacchus*) were selected from the experimental stock of the Biomedical Primate Research Centre (Rijswijk, The Netherlands). Before the monkeys entered the experiment a full physical, hematological and biochemical check-up was performed. Individual data of the monkeys, which were identified with an implanted transponder, are summarized in table 1.

Ethical regulations limit the total blood volume that can be collected and the frequency of MR imaging. Hence, the monkeys were randomly paired and assigned to the groups receiving placebo or antibody treatment before the immunization (see table 1). Paired individuals were handled identically throughout the experiment. The time points for collection of larger blood volumes for immunological tests and MR imaging were determined on the basis of the clinical stage of EAE in one of both individuals of each pair.

During the experiments, the monkeys were individually housed in spacious cages with padded shelters provided at the bottom of the cage and were under constant veterinary care. The daily diet consisted of commercial food pellets for New World monkeys (Special Diet Services, Witham, Essex, England), supplemented with rice, raisins, peanuts, marshmallows, biscuits and fresh fruit. Drinking water was provided *ad libitum*. According to the Dutch law on animal experimentation, the protocol of this study was reviewed and approved by the Institute's Animal Care and User Committee.

*Disease induction and clinical read-out*
EAE was induced by a single immunization with 300 µl human myelin in water (10 mg/ml) emulsified with 300 µl CFA (Difco Laboratories, Detroit, MI) under ketamin anesthesia (6 mg/kg; AST Farma, Oudewater, The Netherlands) as described previously (51). *Bordetella pertussis* was not used for reasons discussed elsewhere (51).

Twice daily clinical signs of EAE were scored blind by a trained observer using a previously described semi-quantitative scale (51): 0, no clinical signs; 0.5, apathy, loss of appetite and altered walking pattern without ataxia; 1, lethargy and/or anorexia; 2, ataxia, sensory loss/blindness; 2.5, hemi- or paraparesis; 3, hemi- or paraplegia; 4, quadriplegia; 5, spontaneous death attributable to EAE. Body weights were determined once weekly as a surrogate disease marker. Monkeys were sacrificed for ethical reasons once a monkey had reached EAE score 3.0, or at day 86 after immunization, being the planned end-point of the study.

*Reactivity, dosing regimen, plasma levels, biodistribution and immunogenicity of anti-IL-12p40 antibody*

The neutralization of marmoset IL-12 by the anti-IL-12p40 antibody was tested using lipopolysaccharide (LPS) stimulated cells. Briefly, plastic adherent cells from common marmosets and human PBMC were stimulated for 24 h with LPS (1 µg/ml). Cell free supernatant was collected and titrated onto 4 day PHA-stimulated human T-cell blasts. After 24 h, IFN-γ levels were determined using standard ELISA techniques. An EC₅₀ stimulation level was determined for each preparation and used at this concentration for titration of antibody to determine the IC₅₀ of neutralization.

The animals were treated with antibody between day 14 and day 86 after immunization (a.i.). Five animals received once weekly i.v. injections of 10 mg/kg antibody in saline into the *vena saphena* under ketamin anesthesia. Control animals (n=5) received once weekly i.v. injections with sterile PBS (1 ml/kg) as placebo treatment.

At 3 days after each dosing, serum was collected and stored frozen until determination of antibody levels with ELISA. Briefly, recombinant IL-12 (10 µg/ml) was coated on 96-well plates (Costar, New York, NY). Thawed serum samples were incubated for 1 h and, after washing, antibody was detected by anti-Fc Ab(ATCC, Manasas, VA). The Ab was purified via protein A, coupled with sulfo-N-hydroxysuccinimide LC biotin (Pierce, Rockford, IL), and detected using horseradish peroxidase-conjugated streptavidin (SA-HRP; Jackson ImmunoResearch Laboratories, West Grove, MA). Concentrations were calculated using a standard curve. Anti-Ab levels were determined by a double antigen EIA using bound anti-IL-12p40 antibody, detected with biotinylated antibody and SA-HRP as described above.
Magnetic Resonance Imaging

High-resolution MRI experiments were performed on a 4.7 T horizontal bore Varian NMR spectrometer (Varian, Palo Alto, CA), equipped with a high-performance gradient insert (11 cm inner diameter, maximum gradient strength 220 mT/m). For in vivo MRI, animals were anesthetized with 30 mg/kg ketamin in combination with 1 mg/kg valium (Diazepam; Kombivet BV, Etten-Leur, The Netherlands). MRI data sets were collected for T1-weighted (T1-w) and T2-w images. A bird cage volume coil (diameter 9 cm) was used for radio frequency transmission and signal reception. For all sequences the field of view was 4x4 cm (matrix 128x128; zero filled to 256x256; in plane resolution 312x312 μm). Post-contrast T1-w images were made after i.v. injection of 0.3 mmol/kg gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA; Magnevist, Schering AG, Berlin Germany). Post-mortem high contrast T2-w images were made of formalin-fixed brains. A solenoidal-coil (4 windings; diameter 35 mm) was used for radio frequency transmission and signal reception (field of view 3x3 cm; matrix 128x128; zero-filling 256x256; in plane resolution 240x240 μm). The in vivo and post-mortem MRI scores were calculated as proposed by Jordan and coworkers (51). More specific: score 0 = no visible lesions; score 1 = one to five lesions on T2 scans; score 2: six to ten lesions on T2 scans; score 3 = 11 to 20 lesions on T2 scans; score 4: more than 21 lesions or diffuse white matter abnormalities on T2 scans. A score 0.5 is added for one gadolinium-enhancing lesion and a score 1 for two or more enhancing lesions. It should be emphasized here that this scoring is based on the number of lesions, rather than that they provide information on the size or the pathomorphological aspects. The data sets were analyzed on an Apple Macintosh I-MAC G3 (Apple Computer, Cupertino, CA) using the public domain National Institute of Health Program (NIH Image 1.52).

Neuropathological examination

After formalin fixation, parts of the brain, spinal cord and peripheral nerves were embedded in paraffin and processed as described previously (29). In brief, the cerebrum and cerebellum were divided into seven or eight coronally cut parts and the spinal cord was dissected transversely. The extent of inflammation, demyelination and axonal pathology was evaluated on 3-5 μm tissue sections stained with hematoxylin and eosin (HE) to visualize infiltrated cells, Klüver Barrera luxol fast blue (LFB) combined with periodic acid schiff (PAS) for myelin and myelin degradation products, and with Bielschowsky silver impregnation for axons. The degree of inflammation was expressed as an index, representing the average number of inflamed blood vessels per spinal cord section (n = 10 to 15 sections). Furthermore, the surface area of demyelination was quantified on 10 to 15 spinal cord fields using a monomorphing grid. Macrophages were visualized using mouse anti-human mAb MRP14 (BMA Biomedicals, Augst, Switzerland), while mAb M4 was used to detect amyloid precursor protein (APP; Boehringer Mannheim, Mannheim, Germany).
Expression profiles of pathogenic effector molecules in the CNS

Immunohistochemistry was performed essentially as previously described (33, 53) with minor modifications. Frozen sections of 6 μm thickness were cut, thaw-mounted on glass slides, and kept overnight at room temperature (RT) in humidified atmosphere. After air-drying for 1 h, slides were fixed in fresh acetone containing 0.02% H₂O₂ (v/v), air-dried for 10 min, washed with PBS, and incubated overnight at 4°C with primary Ab. Incubations with secondary and tertiary reagents were performed for 1 h at RT. Between the incubation steps slides were washed twice with PBS. Detection of primary unlabeled mouse Ab was performed with rabbit anti-mouse Ig HRP (Dako, Glöstrup, Denmark), or in the case of a three-step staining with rabbit anti-mouse Ig biotin (Dako) and HRP labeled avidin-biotin-complex (ABC/HRP; Dako). Rabbit polyclonal Ab was detected with biotin labeled donkey-anti-rabbit-Ig (Amersham, Little Chalfont, United Kingdom) as a second step. HRP activity was revealed by incubation for 10 min at RT with 3-aminio-9-ethyl-carbazole (AEC; Sigma, Zwijndrecht, The Netherlands), resulting in a bright red translucent precipitate.

For detection of IFN-γ and TNF-α, mAb MD-2 and 61E71 were used respectively (U-Cytech, Utrecht, The Netherlands). MAb against IL-4 and IL-6 were obtained from Genzyme (Cambridge, MA). C8.6, a mouse Ab for the detection of IL-12p40, was from Pharmingen (San Diego, CA). IL-10 was visualized using the B-S10 mAb (Instruchemie, Hilversum, The Netherlands). The Ab against IL-18, M318, was obtained from R&D Systems (Abingdon, United Kingdom). 2D9, a mouse mAb against matrix metalloprotease 9 (MMP-9; gelatinase B), was a kind gift from Dr. G. Opdenakker (REGA Institute, Leuven, Belgium). Rabbit polyclonal Ab against CD3 and iNOS were from Dako and Calbiochem (San Diego, CA), respectively. Finally, CD40 was detected using a mouse anti-human CD40 mAb (mAb 5D12; Tanox Pharma Inc., Houston, TX) known to be crossreactive with marmoset CD40 (29, 33).

In situ detection of i.v administered anti-IL-12p40 antibody.

To determine whether intravenous antibody traverses the blood-brain-barrier and gains access to the lesions in the CNS, 1 mg of biotinylated anti-IL-12p40 antibody was i.v. injected into a PBS-treated monkey with an EAE score of 3.0 (Mi-031). At 1 h after injection of the antibody the monkey was sacrificed. Frozen tissue sections of brain and spleen were incubated with SA-HRP (Jackson) for 1 h at RT, followed by tyramide signal amplification (TSA; NEN Life Science Products, Boston, MA). HRP-activity was revealed as described above. To assess whether the biotinylated antibody bound to IL-12p40 produced by astrocytes, tissue sections were double stained using the C8.6 mAb against IL-12p40 and donkey-anti-human polyclonal Ab (Sambio) directed towards glial fibrillary acidic protein (GFAP) characteristic for astrocytes. A combination of HRP and alkaline phosphatase (AP)-labeled
conjugates was used, giving a red precipitate for AEC and a bright blue precipitate using Fast Blue BB base and naphthol AS-MX phosphate for AP, as described in detail previously (33, 53).

**T- and B-cell functions**

Just prior to necropsy, heparinized venous blood was drawn via a needle puncture from the *vena saphena*, after which PBMC were isolated using lymphocyte separation medium (LSM, ICN Biomedical Inc., Aurora, OH). Cell suspensions were prepared from aseptically removed lymph node (LNC) and spleen (SC) and cultured in the presence of recombinant human myelin oligodendrocyte glycoprotein (rhMOG; 10 μg/ml) or human myelin basic protein (hMBP; 25 μg/ml) (47).

Serum was isolated from venous blood collected without coagulant. Sera were collected from paired animals at the pre-immunization stage, when one of both monkeys in a pair displayed EAE score 2.0, and at the time of necropsy. Standard ELISA assays were used to determine IgM and IgG Ab levels directed against MBP and MOG as described (29).

**Statistics**

The χ²-test was used to determine statistically significant treatment-related effects on the progression to EAE score 3.0; Mann-Whitney-*U* test for anti-MOG and anti-MBP Ab levels and for MRI-scores. In all cases, p<0.05 was considered statistically significant.

**Results**

**Effect of in vivo neutralization of IL-12p40 on clinical EAE**

The EAE course in placebo- and antibody-treated monkeys are given as the days of disease onset, when animals showed clear neurological signs (EAE score 2.0), and when the disease score of 3.0 was reached (table I). The percentages of maximal weight loss during the experiment are depicted in the same table. The results show a beneficial effect of antibody treatment on both aspects of clinical EAE. Four PBS-treated monkeys developed severe progressive EAE and one had a period of mild EAE during the observation period of 86 days. In the antibody-treated group, only one monkey, Mi-019, developed clinical signs of EAE (p<0.001 vs. PBS treatment; χ²-test). It should be noted, however, that the time interval between disease onset and EAE score of 3.0 in this animal was considerably longer than in the placebo-treated monkeys (see table I).

**Cross-reactivity, serum levels, and anti-Ab responses**

As shown in results, the IFN-γ inducing properties of conditioned medium derived from LPS-stimulated marmoset adherent cells were neutralized by anti-IL-12p40 antibody. The IC₅₀ was comparable with conditioned media from human cells. The antibody levels measured at 3 days after
each administration were generally maintained at a concentration of 50 to 75 μg/ml throughout the observation period of 86 days. This is approximately a 50-fold excess of the IC50 determined \textit{in vitro}. In one animal (Mi-026) an unexplained disappearance and subsequent reappearance of antibody was observed. However, no anti-Ab responses were detectable, and no alterations in hematological and biochemical parameters were observed during the study period.

\textit{In vivo and postmortem MRI}

The \textit{a priori} condition set before the experiment was to subject paired monkeys to MRI analysis once one of the animals had reached EAE score 2.0 (ataxia), irrespective of the clinical condition of the second monkey. Because of the acute onset of the disease in Mi-032 and Mi-043, both animals had to be euthanized for ethical reasons before an \textit{in vivo} MRI could be made.

The characteristic feature of myelin-induced EAE is a strong periventricular inflammatory reaction that appears as a hyperintense area in T2-w brain MR-images, which is hypointense on T1-w images. Representative examples for both test groups for MRI-detectable changes in the brains of pair 4 found that in monkey Mi-031, which had EAE score 2.0 at the time of scanning, large hyperintense areas were found around the ventral horns of both lateral ventricles. Such abnormalities, which are typical for this model, were completely absent in the clinically normal antibody-treated monkey, Mi-024, which was scanned on the same day had scores of \textit{in vivo} and post-mortem MRI are given in tabular form. The table shows that post-mortem MRI scores of antibody-treated animals tended to be lower when compared to the scores of control animals (p=0.08; Mann-Whitney \textit{U}-test).

\textit{Neuropathology}

All PBS-treated control animals displayed cellular infiltrates in the brain and spinal cord. Four PBS-treated animals showed extensive demyelination which was mainly localized in the spinal cord, while in the animal with the mild EAE (Mi-038) the CNS pathology was mainly localized in the brain (see table I). Although inflammation and demyelination of the CNS were detected in two antibody-treated individuals, three animals (Mi-003; Mi-023; Mi-026) were completely devoid of CNS pathology in the spinal cord and the brain (p=0.03 for inflammation and demyelination; Mann-Whitney \textit{U}-test). Monkey pair 2 as representative example had results showing that the PBS-treated animal Mi-032 displayed large areas with infiltrated mononuclear cells and specific demyelination of the CNS in the spinal cord, while no such pathological changes were observed in the antibody-treated animal of this pair, Mi-026. Macrophages actively involved in demyelination were present in the lesions, while B- and T-cells also could be detected. The immunoreactivity for APP points at degeneration of the demyelinated axons.
CNS lesion load and expression of pathogenic molecules

Immunological aspects of the brain lesions were assessed in cryosections on the basis of the number and size of the infiltrates as defined by hematoxylin counter staining, as well as the expression of the pan-T-cell marker CD3, and of acid phosphatase as a marker of infiltrating macrophages. Both the number and the size of infiltrates in the brains of antibody-treated animals were markedly reduced compared to control animals (Table I). While CD3⁺ T-cells were present in the cellular infiltrates of all EAE affected monkeys, these were undetectable in the brains of three out of four antibody-treated animals that remained asymptomatic. Together these data indicate that neutralization of IL-12p40 affects recruitment of mononuclear cells from the circulation into the CNS.

Next we assessed whether IL-12p40 neutralization would interfere with CNS expression of Th1 or Th2 cytokines (IFN-γ, IL-4, IL-10, IL-12p40, IL-18, TNF-α) or the inflammatory mediators iNOS, and MMP-9. IL-12p40 was expressed by some mononuclear cells within brain infiltrates, and by many astrocytes. The number of IL-12p40-expressing astrocytes decreased with distance from the infiltrates. IL-12p40 was similarly expressed in both groups of animals. This could be expected, since mAb treatment captures released IL-12p40 but likely does not interfere with intracellular synthesis of this factor. Note that in naïve animals or after immunization with ovalbumin emulsified in CFA no IL-12p40 was detectable within the CNS.

The staining is specific, as controls omitting the specific antibody step were shown to be negative. Mononuclear cell infiltrates in the brain of PBS-treated animals displayed clear expression of IL-4, TNF-α and MMP-9. In asymptomatic antibody-treated animals the CNS expression of IL-4, TNF-α and MMP-9 was substantially reduced. IFN-γ was also expressed at lower frequency in non-affected mAb-treated animals (data not shown). The two antibody-treated animals that showed CNS inflammation with histology displayed staining patterns similar as the EAE-affected animals in the PBS-treated group. No marked effect of the antibody treatment on the numbers of IL-6, IL-10, IL-18, and iNOS expressing cells was observed.

Access of anti-IL-12p40 antibody to lesions within the CNS

We assessed whether intravenously administered antibody extravasates into CNS lesions, allowing the capture of locally produced IL-12p40. To this end, biotinylated anti-IL-12p40 antibody was injected i.v. into two PBS-treated animals with clinically manifest EAE (score 3.0) at 1 h before sacrifice. The subsequent immunohistochemical analysis revealed granular staining adjacent to the ventricles, as well as in the cellular infiltrates within the brain of animals injected with the antibody. No such staining was detected in the brains of naïve animals, or ovalbumin/CFA-immunized marmosets. Biotinylated antibody was found attached to the astrocytes in the immediate environment of the inflammatory
infiltrates. This is in conformity with the immunohistochemical demonstration of IL-12p40 by brain astrocytes in EAE-affected animals.

**Anti-myelin T-cell reactivity**

To investigate the effect of anti-IL-12p40 antibody on anti-myelin T-cell activity, we determined the proliferative responses of PBMC, SC and LNC against rhMOG and hMBP in antibody- and placebo-treated animals. The results show that in all cases the T-cell response towards MBP was low, while high responses were found against rhMOG (MOG vs. MBP: p<0.01; Mann-Whitney U-test). In the animals which developed EAE relatively early after disease induction, significant proliferative responses to rhMOG were detectable in SC as well as LNC. In contrast, in all animals sacrificed at the end-point of the study (both PBS- and antibody-treated animals), proliferative responses were mainly restricted to the spleen, while those in LNC were very low or undetectable. As a similar profile was found in the placebo monkey of pair 5, we assume that the number of autoreactive T-cells in the spleen is not affected by antibody treatment.

**Anti-myelin B-cell reactivity**

A critical role of anti-MOG Ab in the marmoset EAE model, mediating demyelination in particular, has been reported (45, 54-57). We therefore determined the serum levels of anti-MBP and anti-MOG Ab in PBS- and antibody-treated animals using ELISA. Anti-MOG and anti-MBP IgM Ab were hardly detectable in the sera of the animals from both groups at any of the evaluated time points. The results also showed that increased anti-MOG (A) and anti-MBP (B) IgG serum levels were found in PBS-treated animals at EAE-score 2.0 when compared with IgG Ab levels in sera from the paired antibody-treated animals (p=0.05; Mann Whitney U-test). At necropsy, only serum levels of MBP-specific IgG were significantly reduced in the asymptomatic antibody-treated animals (p=0.03). Taken together, neutralization of IL-12p40 seems to have a suppressive effect on the production of auto antibodies.

**Discussion**

In this article, we report that neutralization of IL-12p40 results in profound protection from EAE in the common marmoset. While the role of IL-12p40 has been demonstrated in murine EAE, this is the first report that IL-12p40 plays an essential role in the neurological model that most closely represents human chronic MS.

An important result from our study is that the marmosets did not develop a neutralizing Ab response towards peripherally administered Ab. Although the induction of anti-idiotypic Ab cannot be excluded, the remarkably stable serum levels of antibody throughout the treatment period of 72 days strongly indicate that the mAb is not or only marginally immunogenic in vivo. In the past years we have tested a variety of humanized antibodies in non-human primate models of autoimmune arthritis and
encephalomyelitis. Although by extensive engineering often up to 99% of the original mouse back bone had been replaced with human Ig sequences the neutralizing immune response towards the remaining 1% mouse part of the molecule formed a limiting factor for a long-standing therapeutic effect. For example, treatment of EAE-affected marmosets with a chimeric anti-CD40 antibody resulted in substantial neutralizing Ab activity within two weeks of the first administration (29). Similar findings have been published by other groups (58, 59, 60). All PBS-treated monkeys in this study developed clinical EAE. In contrast, four out of five antibody-treated animals remained asymptomatic. The disease progression in the one antibody-treated animal that developed clinical EAE was substantially delayed compared to the PBS-treated monkeys. Our results also show a diminution of MRI-detectable changes in the CNS white matter in antibody-treated animals, when compared to the paired placebo-treated monkeys. A clear effect of the treatment was that the periventricular inflammatory reactions, as observed in all PBS-treated monkeys, were absent in all anti-IL-12p40 antibody treated monkeys.

Interestingly, we observed that antibody treatment did not have a clear beneficial effect on the overall in vivo MRI score. However, it is important to note that T2-w MR images are very sensitive to water. The fact that all pathological changes in the CNS are associated with altered tissue distribution of water explains that each lesion stage in the common marmoset EAE model has a similar appearance on T2-w images (51). Thus, more sophisticated MRI parameters are needed to reveal the beneficial effects of IL-12p40 neutralization in vivo. Quantitative MRI parameters are now being developed and implemented for serial imaging, in particular T1-, T2- and MTR maps (manuscript in preparation). That antibody treatment positively affects structural alterations of CNS white matter was visualized in high contrast T2-w images made of fixed brains. This post-mortem MRI analysis shows clearly reduced MRI scores in the antibody-treated monkeys (with the exception of Mi-019) compared to the placebo group (table I). The beneficial effect of anti-IL-12p40 antibody was confirmed with histology; three out of five antibody-treated monkeys remained completely devoid of CNS infiltration by CD3⁺ T-cells, while inflammation and demyelination were also prevented.

IL-12 is expressed at sites where T-cells and APC interact (8-10). In the common marmoset EAE model, such sites are the secondary lymphoid organs and the developing lesions within the CNS white matter (33, 53). Although microglia are an important source of IL-12 within the CNS (10, 61), our results demonstrate that astrocytes also express intracellular IL-12p40.

Intravenously injected biotinylated antibody was retrieved at peripheral (spleen/lymph nodes) as well as central (brain) locations, confirming our previous finding that during active disease the blood-brain-barrier is permeable for large molecules like IgG (29). This allows the local action of antibody within the CNS at locations where infiltrating T-cells interact with resident APC or infiltrating macrophages.
As the time needed for 1 min diffusion of a 20kD protein into unaffected CNS parenchyma has been estimated at about 3 days (62), we assume that the activity range of i.v. injected antibody (±50 kD) inside the CNS is limited to the perivascular space (Virchows Robin space) and the lesion.

In view of the 100% susceptibility of outbred common marmosets to myelin-induced EAE (7, 45) it is highly unlikely that the observed effects of anti-IL-12p40 antibody could be explained by the possibility that the antibody-treated monkeys that remained asymptomatic were all non-responders to the disease induction. Hence, the conclusion is warranted that antibody treatment protects marmoset monkeys immunized with human myelin in CFA against the clinical and neuropathological expression of EAE. Our present results suggest that anti-IL-12p40 antibody may modulate autoimmune responses.

Anti-myelin Ab are considered a critical factor in the marmoset EAE model as mediators of CNS demyelination (45, 54-57). It is therefore of great interest that during development of EAE, circulating auto Ab levels were consistently lower in anti-IL-12p40 antibody-treated animals than in the paired PBS-treated animals. This suggests that besides preventing T-cell recruitment into the CNS, auto Ab responses are also mediated by IL-12p40. Similar observations have been made in marmosets that were protected from EAE by anti-CD40 mAb treatment. In that study the protective effect of the mAb to clinical EAE was associated with abrogation of broadening of the epitope response against MOG (29).

The different reactivity patterns of T-cells present in PBMC, LNC and SC to MBP and MOG between PBS- and antibody-treated monkeys can be explained by the different disease duration. Our data are in line with observations by Targoni and coworkers (64) that T-cell autoreactivity in mice wanes in time from draining lymph nodes and the circulation during EAE development, but persists in the spleen. All antibody-treated monkeys displayed a similar reaction pattern as the one PBS-treated monkey sacrificed at the same time point (day 86). We assume therefore that IL-12p40 modulates the development of autoreactive T-cells, yet does not interfere with cell circulation kinetics.

A likely result of IL-12p40 neutralization is deviation of the encephalitogenic myelin-reactive T-cells into the Th2 direction as was observed in mice (21). However, we are presently unable to prove this since reagents for specific detection of Th2 cytokines in common marmosets with ELISA are lacking. Instead, using immunostaining we determined cytokine expression patterns within the CNS of both groups of monkeys.

In three out of five antibody-treated animals, neither T-cell infiltrates nor IFN-γ or IL-4 producing cells could be detected. Moreover, inflammatory mediators like TNF-α and MMP-9 were not expressed in the brains of these monkeys. In contrast, in all PBS-treated animals we found brain infiltrates expressing each of the tested inflammatory mediators. The patterns of intracellular IL-12p40 expression within the CNS white matter did not differ between PBS- and antibody-treated animals.
In conclusion, anti-IL-12p40 antibody has direct access to secondary lymphoid organs and the CNS. Since antibody treatment was initiated at a late stage (day 14 after immunization), it is less likely that antibody interferes with initial activation of autoreactive T- and B-cells. However, by neutralization of APC-derived IL-12, it affects the influx of autoreactive T-cells into the CNS, inhibits the inflammatory response, and suppresses the auto Ab response against myelin proteins resulting in prevention of EAE development.

References


Abbreviations used in this paper: ABC/HRP: horseradish peroxidase labeled avidin-biotin-complex; AEC: 3-amino-9-ethyl-carbazole; a.i.: after immunization; APP: amyloid precursor protein; EAE: experimental autoimmune encephalomyelitis; Gd-DTPA: gadolinium-diethylenetriaminepentaacetic acid; GFAP: glial fibrillary acidic protein; HE: hematoxylin and eosin; LNC: lymph node cells; LFB: luxol fast blue; LSM: lymphocyte separation medium; iNOS: inducible nitric oxide synthase; (h)MBP: (human) myelin basic protein; MMP-9: matrix metalloprotease 9; MRI: magnetic resonance imaging; MS: multiple sclerosis; (rh)MOG: (recombinant human) myelin oligodendrocyte glycoprotein; PAS: Periodic Acid-Schiff; ROI: region of interest; RT: room temperature; SA-HP: HRP conjugated streptavidin; SC: spleocytes, spleen cells; T1-w: T1-weighted; TSA: tyramide signal amplification

Example 4: Comparison of the therapeutic efficacy of anti-IL-12p35 and anti-IL-12/23p40 antibodies in murine experimental autoimmune encephalomyelitis (EAE)

Summary

This set of studies was performed to investigate the therapeutic efficacy of IL-12 or IL-12/23 specific neutralization in a mouse model for multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Neutralizing rat anti-mouse monoclonal antibodies (mAbs) specific for the p35 subunit of IL-12 or the p40 subunit that is shared between IL-12 and IL-23 were administered either prior to disease induction, prior to disease onset, or after disease was ongoing. In all cases, only anti-p40 demonstrated therapeutic potential. These data suggest that IL-23 is the predominant contributor to disease pathogenesis in this autoimmune model.

Abbreviations

<table>
<thead>
<tr>
<th>IL</th>
<th>Interleukin</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>Th</td>
<td>T helper cell</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>cs</td>
<td>Clinical score</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetics</td>
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Introduction

Biologically active IL-12 exists as a heterodimer comprised of 2 covalently linked subunits of 35 (p35) and 40 (p40) kilo Daltons. Several lines of evidence have demonstrated that IL-12 can induce robust Th1 immune responses that are characterized by production of IFNγ and IL-2 from CD4+ T cells. Inappropriate Th1 responses, and thus IL-12 expression, are believed to correlate with many
autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, insulin-dependent diabetes mellitus, and uveitis. In animal models, IL-12 neutralization was shown to ameliorate autoimmune disease. However, these studies neutralized IL-12 through its p40 subunit. The recent description of IL-23 (1), a heterodimeric cytokine that shares the p40 subunit, made it important to determine whether previous findings were due to IL-12 or IL-23 activity. Therefore, we compared p35 and p40 specific neutralization in a mouse model of autoimmunity, experimental autoimmune encephalomyelitis (EAE). Neutralizing antibodies specific for IL-12p35 had no effect on EAE progression. In contrast, neutralization of both IL-12 and IL-23 with an anti-p40 mAb suppressed clinical signs of EAE whether antibody was administered before or after Th1 differentiation. Our data suggests that the activity of anti-p40 treatment in EAE is based solely on neutralization of IL-23.

Methods and Materials

Animals:
Female C3H/HEB/FEJ mice (Jackson Laboratories, Bar Harbor, ME) were used in pharmacokinetic analyses. For EAE studies, female B10.PL (H-2b) mice were purchased from the Jackson Laboratories, and were used between 6-8 weeks of age. All animals were maintained according to IACUC guidelines under approved protocols.

Antibodies:
C17.8 (rat anti-mouse IL-12/23p40, IgG2a), and C18.2 (rat anti mouse IL-12p35, IgG2a) hybridomas were generous gifts of Dr. Giorgio Trinchieri and the Wistar Institute (Philadelphia, PA).

Ascites was generated at Harlan Bioproducts (Indianapolis, IN) and purified by protein G affinity.

Serum PK of rat anti-mouse antibodies:
Female C3H/HEB/FEJ mice, approximately 20-25 grams, were individually weighed and treated with a single 5 mg/kg intraperitoneal dose of $^{125}$I labeled antibody (C17.8, C18.2), with a constant dose volume/mouse of 10 mL/kg. Retro-orbital bleeds were taken from anesthetized mice at 30 minutes, 6 and 24 hours, 4, 7, 11 and 18 days. Blood samples were allowed to stand at room temperature for at least 30 minutes, but no longer than 1 hour, and were then centrifuged at approximately 2,500-3,500 rpm for 10-15 minutes. Approximately 50 uL aliquots of each serum sample were counted for $^{125}$I using a LKB Compugamma 1282 counter (Wallac, Gaithersburg, MD). 10 uL aliquots of the injectates were also counted. The average fraction of injected counts at each time point was calculated and multiplied by the total uL of antibody injected to determine the total uL remaining in the serum at each time point. Data is shown as the mean ± s.d. with 5-10 animals in each group.

EAE Induction and Scoring:
For EAE induction, female B10.PL mice were injected subcutaneously over four sites on the back with a total of 100 µL of CFA (containing 200 µg Mycobacterium tuberculosis Jamaica strain)
combined with 200 µg guinea pig-MBP (Sigma). Mice also received 200 ng pertussis toxin (List Biological, Campbell, CA) i.p. in 0.2 ml PBS at the time of immunization and 48 hours later. Mice received i.p. injections of C17.8 (anti-IL-12p40) or C18.2 (anti-IL-12p35) monoclonal antibodies diluted to 100 mg/kg (C18.2) or 20 mg/kg (C17.8) in PBS, on indicated days. Control mice received PBS or Rat IgG (Biosource) at 20 mg/kg in PBS.

Animals that demonstrated clinical signs (cs) were scored as follows: limp tail or waddling gait with tail tonicity 1, waddling gait with limp tail (ataxia) 2, ataxia with partial limb paralysis 2.5, full paralysis of one limb 3, full paralysis of one limb with partial paralysis of second limb 3.5, full paralysis of two limbs 4, moribund 4.5, death 5. Animals that scored a 5 were not included in the mean daily cs analysis for the rest of the experiment. Daily cs are averaged for the group, and mean incidence, day of onset, highest acute cs, cumulative cs, cs/day, number of relapses and relapse severity ± sem are described. Mean cumulative cs per group was calculated by averaging the sum of daily clinical scores for individual animals. Cs/day was calculated by dividing the cumulative cs by the number of days the animal remained in the study. To determine the mean day of onset, animals not developing EAE were not included in the analysis. To determine the mean highest cs, mice not developing EAE were assigned a value of “0” and included in the analysis. Relapses were defined by a full point drop in clinical score sustained for at least 2 observed days followed by a full point increase in clinical score sustained for at least 2 observed days.

Results and Discussion

Anti-p35 and anti-p40 mAbs have identical pharmacokinetics

To establish the clearance rates of anti-p40 and anti-p35 antibodies, normal mice were injected with a single 5 mg/kg dose of ¹²⁵I labeled antibodies and circulating levels were measured for 11 days post antibody administration. Anti-p35 and anti-p40 had overlapping pharmacokinetics, demonstrating that clearance rates are identical in normal mice (2). The expected clearance rate of each mAb is approximately 7-10 days. Although this is a single dose PK study, these data support once weekly dosing for in vivo studies.

Only anti-p40 treatment prior to EAE induction is protective.

To determine the relative roles of IL-12 and IL-23 in an autoimmune disease, we utilized a murine model for multiple sclerosis, relapsing experimental autoimmune encephalomyelitis (EAE). Upon EAE induction with myelin basic protein (MBP) in adjuvant, B10.PL mice typically exhibit an initial episode of paralysis (acute disease), then recover either partially or completely and progress through multiple relapses and/or chronic EAE. It has long been assumed that EAE is dependent upon IL-12 expression since IL-12 is believed to be a primary mediator of Th0 to Th1 differentiation.
However, to distinguish the potential role of IL-23 in EAE induction, neutralizing concentrations of anti-p40 (IL-12 and IL-23) or anti-p35 (IL-12 only) antibodies were established one day prior to immunization for EAE (Day -1). Onset of disease can vary between animals; therefore, treatment was repeated 7 and 14 days later to ensure that anti-p35 and IL-p40 antibodies were present during Th1 differentiation. Several in vitro neutralization studies have demonstrated that the anti-40 mAb is 5 times more effective in neutralizing IL-12 than the anti-p35 mAb (data not shown). Therefore, the dose of anti-p35 mAb was adjusted to be 5 fold higher than anti-p40 in all EAE experiments. In two separate experiments, mice treated with Rat IgG isotype control antibody (20 mg/kg) or anti-p35 (100 mg/kg) did not demonstrate protection from disease. It is important to note that peripheral administration of a non-specific control antibody (Rat IgG) did not alter the clinical course of disease when compared to non-treated mice with EAE. In both studies, mice treated with anti-p40 mAb (20 mg/kg) exhibited nearly complete inhibition of EAE clinical signs. Remarkably, suppression of disease extended beyond the expected rate of antibody clearance through 70 days post EAE induction. In each experiment, only one animal treated with anti-p40 exhibited two consecutive days of EAE clinical signs, and each demonstrated a late onset and significantly lower acute clinical scores, cumulative clinical scores, and no relapses in disease (Table 1). These results demonstrated that neutralization of IL-12 and IL-23 through the shared p40 subunit provided nearly complete protection from EAE. In contrast, specific neutralization of IL-12 only via anti-p35 was ineffective. These data strongly suggest that EAE is not mediated by IL-12.

Table 1. EAE clinical scores with IL-12 and IL-23 neutralization prior to Th1 differentiation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Mortality</th>
<th>Day of onset</th>
<th>Highest acute cs</th>
<th>Cumul cs</th>
<th>Cs/day</th>
<th>No. relapse</th>
<th>Relapse severity</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>-Rat IgG</td>
<td>13/13</td>
<td>4/13</td>
<td>30.5 ± 3.2</td>
<td>3.6 ± 0.3</td>
<td>71.4 ± 14.1</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>11/13</td>
<td>8/13</td>
<td>29.6 ± 3.4</td>
<td>3.5 ± 0.5</td>
<td>45.3 ± 11.5</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>1/13</td>
<td>0/13</td>
<td>40.0</td>
<td>0.1</td>
<td>1.2 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>P-2001-079</td>
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<tr>
<td>No treatment</td>
<td>6/7</td>
<td>0/7</td>
<td>24.7 ± 2.7</td>
<td>3.2 ± 0.6</td>
<td>110.4 ± 10.4</td>
<td>1.7 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>3.8 ± 0.1</td>
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<td>20.4</td>
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<tr>
<td>Rat IgG</td>
<td>9/9</td>
<td>2/9</td>
<td>29.1 ± 2.9</td>
<td>3.8 ± 0.2</td>
<td>90.6 ± 10.1</td>
<td>1.5 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>10/10</td>
<td>1/10</td>
<td>30.0 ± 2.6</td>
<td>3.9 ± 0.2</td>
<td>94.9 ± 17.8</td>
<td>1.4 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>1/10</td>
<td>0/10</td>
<td>61.0</td>
<td>0.3</td>
<td>1.6 ± 1.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 + 0.0</td>
<td>0.0 + 0.0</td>
</tr>
</tbody>
</table>

\[ a \] Clinical score (cs)

\[ b \] Cumulative cs
EAE induction. Data is shown as the mean per group ± s.e.m.

Legend to table I

Clinical signs of EAE were scored as: 0, no clinical signs; 0.5, apathy, loss of appetite and altered walking pattern without ataxia; 1.0, lethargy and/or anorexia; 2.0, ataxia, sensory loss/blindness; 2.5, hemi-or paraparesis; 3.0, hemi- or paraplegia; 4.0, quadriplegia; 5.0, spontaneous death attributable to EAE. Body weight was determined at the day of dosing as a surrogate disease marker. The maximal weight loss during the experiment is expressed as a percentage of the starting weight. Animals were treated from day 14 after immunization (a.i.) onwards and either sacrificed when a EAE-score 3.0 was reached or at the end of the study period (day 86 a.i.). T1-w (pre- and post-contrast) and T2-w MRI data sets were acquired and scored as described in materials and methods. MRI were made once one of the animals had reached EAE score 2.0 (ataxia), irrespective of the clinical condition of the second monkey. Because of the acute onset of the disease in Mi-032 and Mi-043, both animals were euthanized for ethical reasons before an in vivo MRI could be made. Consequently, the in vivo MRI of Mi-026 and Mi-023 was recorded at day 55 a.i.n.d.: not done. The number of infiltrates in the brain were quantified using immunohistochemistry. The number of infiltrates per section were scored as: -, no infiltrates; +, 1-3 infiltrates; ++, 4-10 infiltrates; +++>, >10 infiltrates. Results represent the mean of two sections. The size of the largest infiltrate found in two sections was scored as: +, small (<30 cells); ++, medium (>30 cells); +++, large (>100 cells). The inflammatory index (Infl. Index) in the spinal cord was quantified as being the average number of inflamed blood vessels per spinal cord cross-section (10 to 15 sections). Furthermore, the surface area of demyelination (Demyel (%) was quantified on 10 to 15 spinal cord cross sections using a monomorphic grid. Inflammation and demyelination in the brain is expressed as present (+) or absent (-).

Only anti-p40 treatment just prior to disease onset is protective.

Although prophylactic treatment completely protected mice from EAE, it remained to be determined if IL-12 specific neutralization would be protective once the Th1 population was established in vivo. Therefore, in a separate set of experiments, mice were treated with either a control antibody (Rat IgG), anti-p35, or anti-p40 monoclonal antibodies ten days after EAE induction, but prior to disease onset. Since typical immune responses occur within 7 days, this time point should reflect the effects of anti-IL-12 or anti-IL-23 mAbs on differentiated Th1 cells. EAE onset can vary between animals, therefore treatment was repeated 7 and 14 days later to ensure that anti-p35 and anti-p40 antibodies were present during the onset of disease. In two separate experiments, mice treated with isotype control antibody (20 mg/kg) or anti-p35 (100 mg/kg) were not protected from disease, when compared to untreated EAE mice. However, mice treated with anti-p40 mAb (20 mg/kg) were
significantly protected from EAE. As shown in the previously described studies, disease suppression was observed well beyond the time required for clearance of peripherally administered antibody through day 70 post EAE induction. Considering that antibody was not administered until after Th1 differentiation (day 10), it was not surprising that disease incidence, day of onset, and the highest clinical score during acute EAE were not different in any group (Table 2). However, in both experiments, mice receiving anti-p40 exhibited significantly lower cumulative clinical scores, clinical scores per day, and relapse severity.

Table 2. EAE clinical scores with IL-12 and IL-23 neutralization after Th1 differentiation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Mortality</th>
<th>Day of onset</th>
<th>Highest acute cs</th>
<th>Cumul cs</th>
<th>Cs/day</th>
<th>No. of relapses</th>
<th>Relapse severity</th>
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<tr>
<td>P-2001-057</td>
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<tr>
<td>No treatment</td>
<td>7/10</td>
<td>0/8</td>
<td>30.6 ± 2.7</td>
<td>3.2 ± 0.5</td>
<td>51.5 ± 14.4</td>
<td>0.8 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>9/10</td>
<td>0/10</td>
<td>25.9 ± 2.7</td>
<td>2.7 ± 0.5</td>
<td>74.7 ± 15.8</td>
<td>1.2 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>9/10</td>
<td>0/10</td>
<td>25.8 ± 2.6</td>
<td>2.5 ± 0.4</td>
<td>58.8 ± 15.6</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>6/10</td>
<td>0/7</td>
<td>34.7 ± 6.3</td>
<td>1.6 ± 0.5</td>
<td>14.9 ± 7.5</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>1.5 ± 0.5</td>
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<tr>
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<tr>
<td>No treatment</td>
<td>8/9</td>
<td>2/9</td>
<td>15.8 ± 2.2</td>
<td>2.1 ± 0.6</td>
<td>56.4 ± 19.1</td>
<td>0.9 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Rat IgG</td>
<td>9/10</td>
<td>4/10</td>
<td>20.0 ± 2.5</td>
<td>3.8 ± 0.5</td>
<td>70.1 ± 17.7</td>
<td>1.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>10/10</td>
<td>1/10</td>
<td>16.5 ± 1.1</td>
<td>3.2 ± 0.3</td>
<td>93.8 ± 15.7</td>
<td>1.4 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>10/10</td>
<td>2/10</td>
<td>13.6 ± 1.1</td>
<td>2.7 ± 0.5</td>
<td>23.2 ± 7.9</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>2.0 ± 0.4</td>
</tr>
</tbody>
</table>

*Clinical score (cs)
 Cumulative cs

Mice were treated on days 10, 17, and 24 and clinical scores were analyzed from day 0 through 70 days post EAE induction. Data is shown as the mean per group ± s.e.m.

Only anti-p40 treatment during established EAE is protective.

The most difficult, but clinically relevant, hurdle for any therapy is to suppress established disease. Therefore another set of experiments was performed in which mice were immunized for EAE, then divided into treatment groups once disease was ongoing. Approximately 30 days post EAE induction, mice had progressed through the acute phase of disease. At this time, animals were divided into groups with comparable cumulative and daily clinical scores. Treatment was repeated 7 and 14 days later to ensure that antibodies were available in neutralizing concentrations during the transition from acute to
chronic or remitting-relapsing disease. Only anti-p40 treatment (20 mg/kg) ameliorated disease when compared to either isotype control antibody (20 mg/kg) or anti-p35 (100 mg/kg) treated animals. Disease suppression was observed through day 80 post EAE induction. In both experiments, analysis from the first day of treatment through day 80 demonstrated that mice receiving anti-p40 exhibited lower cumulative clinical scores, clinical scores per day, and the least highest clinical score post treatment. These data suggest that not only is IL-23 likely to mediate Th1 differentiation (Table 1) and EAE induction (Table 2), but IL-23 also contributes to the effector phase of chronic autoimmune responses (Table 3). Therefore, anti-p40 treatment can offer therapy at any time in the progression of autoimmune disease.

Table 3. EAE clinical scores with IL-12 and IL-23 neutralization during established EAE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Daily cs</th>
<th>Mortality</th>
<th>Cumul cs</th>
<th>Cs/day</th>
<th>Cs</th>
<th>No. of relapses</th>
<th>Relapse severity</th>
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</tr>
<tr>
<td>No treatment</td>
<td>2.7 ± 0.6</td>
<td>1/5</td>
<td>132.9 ± 29.3</td>
<td>3.3 ± 0.3</td>
<td>4.1 ± 0.2</td>
<td>2.4 ± 0.5</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>2.3 ± 0.7</td>
<td>1/5</td>
<td>135.9 ± 16.5</td>
<td>2.7 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>2.0 ± 0.2</td>
<td>1/6</td>
<td>75.6 ± 16.1</td>
<td>1.9 ± 0.3</td>
<td>2.8 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>0.7 ± 0.3</td>
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<tr>
<td>P-2002-093</td>
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</tr>
<tr>
<td>Rat IgG</td>
<td>1.7 ± 0.8</td>
<td>1/5</td>
<td>87.7 ± 16.4</td>
<td>2.1 ± 0.2</td>
<td>3.7 ± 0.4</td>
<td>1.2 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Anti-p35</td>
<td>1.9 ± 0.7</td>
<td>1/5</td>
<td>98.2 ± 9.7</td>
<td>2.2 ± 0.1</td>
<td>3.7 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Anti-p40</td>
<td>2.4 ± 0.8</td>
<td>0/5</td>
<td>71.7 ± 21.6</td>
<td>1.5 ± 0.4</td>
<td>2.9 ± 0.6</td>
<td>0.8 ± 0.5</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

* treatment (Tx)
_b clinical score (cs)
_c cumulative cs

Mice were divided into 3 treatment groups with comparable disease severity once EAE was established (approximately day 30). Clinical scores were analyzed from the first day of treatment through 80 days post EAE induction. Data is shown as the mean per group ± s.e.m.

Conclusions

The understanding of the role of IL-12 in immune function has been based on studies of the p40 subunit of IL-12. Therefore, we conducted a side by side comparison of neutralization of the IL-12 specific p35 subunit versus the p40 subunit shared between IL-12 and IL-23 in an animal model of autoimmune disease. Neutralization via anti-p40 significantly inhibited EAE when mAb was administered at any time point. However, IL-12 specific neutralization was completely ineffective.
Therefore, our data shows that IL-12 only partially contributes to this autoimmune model and that IL-23 is expected to be the more prominent mediator of autoimmune T cell responses.

It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.
WHAT IS CLAIMED IS:

1. An isolated anti-multiple sclerosis Ig derived protein, comprising at least one CDR, wherein said Ig derived protein specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence, selected from the group consisting of at least one of 198-215, 211-219, 226-249, 253-270, 280-290, 293-302, 353-372, 391-401, 405-443, 451-470, 476-478, 486-497 of SEQ ID NO:1.

2. An multiple sclerosis Ig derived protein according to claim 1, wherein said Ig derived protein binds multiple sclerosis with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.

3. An multiple sclerosis Ig derived protein according to claim 1, wherein said Ig derived protein substantially neutralizes at least one activity of at least one multiple sclerosis protein.

4. An isolated nucleic acid encoding at least one isolated anti-multiple sclerosis Ig derived protein according to claim 1.

5. An isolated nucleic acid vector comprising an isolated nucleic acid according to claim 4.

6. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to claim 5.

7. A host cell according to claim 6, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.

8. A method for producing at least one anti-multiple sclerosis Ig derived protein, comprising translating a nucleic acid according to claim 4 under conditions in vitro, in vivo or in situ, such that the multiple sclerosis Ig derived protein is expressed in detectable or recoverable amounts.

9. A composition comprising a multiple sclerosis Ig derived protein according to claim 1 and further comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a multiple sclerosis therapeutics, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug or the like, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a
radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

10. A method for treating a multiple sclerosis related condition in a cell, tissue, organ or animal, comprising
(a) contacting or administering a composition comprising a modulating effective amount of at least one multiple sclerosis Ig derived protein according to claim 1, with, or to, said cell, tissue, organ or animal.

11. A method according to claim 10, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

12. A method according to claim 10, wherein said contacting or said administrating is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabranchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraostral, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intraterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

13. A method according to claim 10, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one selected from at least one multiple sclerosis therapeutic, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antispasmodic, a corticosteroid, an anabolic steroid, a multiple sclerosis related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antifulcer, a laxative, an anticoagulant, an erythropoietin, a filgrastim, a sargramostim, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegie, an alkylating agent, an antimitobolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha, a cytokine or a cytokine antagonist.

14. A method according to claim 13, wherein said multiple sclerosis therapeutic is selected from at least one of beta-interferon 1a, beta-interferon 1b, gultiramer acetate, cyclophosphamide, azathioprine, glucocorticosteroids, methotrexate, paclitaxel, 2-chlorodeoxyadenosine, mitoxantrone, an IL-10, TGBeta, CD4, CD52, antegen, CD11, CD18,
TNF alpha, IL-1, IL-2, and/or CD4 antibody or antibody receptor fusion protein, interferon alpha, immunoglobulin, ismide, insulin-like growth factor-1 (IGF-1), elprodil, pirfenidone, oral myelin

15. A method according to claim 13, wherein said multiple sclerosis therapeutic is selected from at least one compound or protein that acts on one or more of at least one of: autoimmune suppression of myelin destruction, immune regulation, activation, proliferation, migration and/or suppressor cell function of T-cells, inhibition of T cell receptor/peptide/MHC-II interaction, Induction of T cell anergy, deletion of autoreactive T cells, reduction of trafficking across blood brain barrier, alteration of balance of pro-inflammatory (Th1) or immunomodulatory (Th2) cytokines, inhibition of matrix metalloprotease inhibitors, neuroprotection, reduction of gliosis, promotion of re-myelination.

16. A medical device, comprising at least one anti-multiple sclerosis Ig derived protein according to claim 1, wherein said device is suitable to contacting or administering said at least one anti-multiple sclerosis Ig derived protein by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraoveal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intruterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

17. An article of manufacture for human pharmaceutical use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one anti-multiple sclerosis Ig derived protein according to claim 1.

18. The article of manufacture of claim 17, wherein said container is a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraoveal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intruterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

19. A method for producing at least one anti-multiple sclerosis Ig derived protein according to claim 1, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts said Ig derived protein.

20. At least one anti-multiple sclerosis Ig derived protein produced by a method according to claim 19.

21. An anti-idiotypic antibody or fragment that specifically binds an Ig derived protein according to claim 1.
22. An Ig derived protein that competitively inhibits the binding of an Ig derived protein according to claim 1 to a ligand.

23. Any invention described herein.
SEQUENCE LISTING

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MULTIPLE SCLEROSIS DISEASE DERIVED PROTEINS, COMPOSITIONS, METHODS
AND USES

CEN0288

Appl. No. 60/367,896

2002-03-26

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PatentIn Ver 2.0

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PRT

Homo sapiens

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Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys Leu

His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln Lys

Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp

His Glu Asp Ile Thr Lys Asp Thr Ser Thr Val Glu Ala Cys Leu

Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr

Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe

Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met Tyr

Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro Lys

Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu Leu

Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser Ser

Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Leu Cys Ile Leu

Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met Ser

Tyr Leu Asn Ala Ser Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val

Page 1
Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr

Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser

Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu

Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu

Ser His Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser

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Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu

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Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser

Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu

Trp Ala Ser Val Pro Cys Ser

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