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(54) Title: COMPOSITIONS COMPRISING BACTERIAL STRAINS

(57) Abstract: The invention provides compositions comprising bacterial strains for treating and preventing autoimmune and inflammatory disorders of the central nervous system.

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COMPOSITIONS COMPRISING BACTERIAL STRAINS

TECHNICAL FIELD

This invention is in the field of compositions comprising bacterial strains isolated from the mammalian digestive tract and the use of such compositions in the treatment of disease.

BACKGROUND TO THE INVENTION

The human intestine is thought to be sterile *in utero*, but it is exposed to a large variety of maternal and environmental microbes immediately after birth. Thereafter, a dynamic period of microbial colonization and succession occurs, which is influenced by factors such as delivery mode, environment, diet and host genotype, all of which impact upon the composition of the gut microbiota, particularly during early life. Subsequently, the microbiota stabilizes and becomes adult-like [1]. The human gut microbiota contains more than 1500 different phylotypes, dominated in abundance levels by two major bacterial divisions (*phyla*), the Bacteroidetes and the Firmicutes [2-3]. The successful symbiotic relationships arising from bacterial colonization of the human gut have yielded a wide variety of metabolic, structural, protective and other beneficial functions. The enhanced metabolic activities of the colonized gut ensure that otherwise indigestible dietary components are degraded with release of by-products providing an important nutrient source for the host and additional health benefits. Similarly, the immunological importance of the gut microbiota is well-recognized and is exemplified in germfree animals which have an impaired immune system that is functionally reconstituted following the introduction of commensal bacteria [4-6].

The discovery of the size and complexity of the human microbiome has resulted in an on-going evaluation of many concepts of health and disease. Certainly, dramatic changes in microbiota composition have been documented in gastrointestinal disorders such as inflammatory bowel disease (IBD)[7-10]. More recently, there is increased interest in the art regarding alternations in the gut microbiome that may play a pathophysiological role in human brain diseases [11]. Preclinical and clinical evidence are strongly suggesting a link between brain development and microbiota [12].

In recognition of the potential positive effect that certain bacterial strains may have on the animal gut, various strains have been proposed for use in the treatment of various diseases (see, for example, (see, for example, [13-16]). A number of strains, including mostly *Lactobacillus* and *Bifidobacterium* strains, have been proposed for use in treating various bowel disorders (see [17] for a review). Strains of the genus *Blautia* have also been proposed for use in modulating the microbial balance of the digestive ecosystem (WO 01/85187) and particular species have been proposed for use in treating systemic diseases distanced from the gut (WO 2016/203218). However, the relationship between different bacterial strains and different diseases, and the precise effects of particular bacterial strains on the gut and at a systemic level and on any particular types of diseases, are poorly characterised.

The efficacy of *Blautia hydrogenotrophica* to treat or prevent autoimmune or inflammatory disorder of the central nervous system has not yet been demonstrated. Broad classes of commensal microbes have been proposed to exert an effect on autoimmune and inflammatory disorders [18], including the *Blautia* species *Blautia producta* [19]. However, the relationship between the large number of species disclosed in [18] and the different diseases they are proposed to treat has not been demonstrated.

There is a requirement for the potential effects of gut bacteria to be characterised so that new therapies using gut bacteria can be developed.

SUMMARY OF THE INVENTION

The inventors have developed new therapies for treating and preventing autoimmune and inflammatory disorders of the central nervous system. In particular, the inventors have identified that bacterial strains from the species *Blautia hydrogenotrophica* can be effective for treating or preventing autoimmune and inflammatory disorders of the central nervous system. As described in the examples, administration of compositions comprising *Blautia hydrogenotrophica* may reduce severity and incidence of symptoms in a mouse model of CNS inflammation and multiple sclerosis (MS). Therefore, in a first embodiment, the invention provides a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, for use in a method of treating or preventing an autoimmune or inflammatory disorder of the central nervous system.

The bacterial strain in the composition is of *Blautia hydrogenotrophica*. Closely related strains may also be used, such as bacterial strains that have a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica*. Preferably, the bacterial strain has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1. Most preferably, the bacterial strain in the composition is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294.

In preferred embodiments, the composition of the invention is for use in treating or preventing a demyelinating autoimmune disease or an inflammatory demyelinating disease. In particularly preferred embodiments, the composition of the invention is for use in treating or preventing multiple sclerosis. The EAE model studied in the examples is particularly relevant to these diseases and especially MS.

In preferred embodiments, the composition of the invention is for use in a method of reducing disease incidence or disease severity. In further preferred embodiments, the composition is for use in preventing a decline in motor function or for use in improving motor function. The results obtained in the examples demonstrate that the compositions of the invention can be effective for reducing disease incidence and severity and improving motor function.

In certain embodiments, the composition of the invention is for oral administration. Oral administration of the strains of the invention can be effective for treating autoimmune or inflammatory disorders of the central nervous system. Also, oral administration is convenient for patients and practitioners and allows delivery to and / or partial or total colonisation of the intestine.

In certain embodiments, the composition of the invention comprises one or more pharmaceutically acceptable excipients or carriers.

In certain embodiments, the composition of the invention comprises a bacterial strain that has been lyophilised. Lyophilisation is an effective and convenient technique for preparing stable compositions that allow delivery of bacteria.

In certain embodiments, the invention provides a food product comprising the composition as described above.

In certain embodiments, the invention provides a vaccine composition comprising the composition as described above.

Additionally, the invention provides a method of treating or preventing to an immune or inflammatory disorder of the central nervous system, comprising administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1a: EAE clinical scores from day 0 to day 30. **Figure 1b.** EAE clinical scores from day 0 to day 35.

Figure 2a: Area under the curve (AUC) analysis of EAE clinical scores from day 0 to day 30. **Figure 2b:** Area under the curve (AUC) analysis of EAE clinical scores from day 0 to day 35.

Figure 3: Spinal cord and brain histopathology score analysis. Data shows mean ± SEM. * $p < 0.05$ and *** $p < 0.001$ vs. vehicle (PBS) group.

Figure 4: Representative pictures of spinal cord sections stained with haematoxylin and eosin.

Figure 5: Representative pictures of brain sections stained with haematoxylin and eosin.

Figure 6: Effect of *Blautia hydrogenotrophica* (10^{10} / day for 14 days) on short chain fatty acids production (RMN ^{1}H) in caecal contents of healthy HIM rats.

Figure 7: qPCR evaluation of *B. hydrogenotrophica* population in faecal samples of IBS-HMA rats treated or not with a composition comprising *B. hydrogenotrophica* (BlautiX) for 28 days.

Figure 8: Short chain fatty acids (SCFA) concentrations in caecal samples of IBS-HMA rats treated or not with *B. hydrogenotrophica* (Blautix) for 28 days. **Figure 8A** shows concentration of total SCFA. **Figure 8B** shows concentration of Acetic acid, Propionic acid and Butyric acid.

Figure 9: Levels of IgG antibody response to MOG 35-55 peptide in Day -14 Serum (Figure 9a) and in Day 35 Serum (Figure 9b). Bar represents median (n=12 unless animals were terminated earlier than day 35 end-point). * $p < 0.05$.

Figure 10: Levels of splenocyte proliferation to MOG 35-55 peptide. Data shows mean ± SEM (n=12 unless animals were terminated earlier than day 35 end-point).

DISCLOSURE OF THE INVENTION

Bacterial strains

The compositions of the invention comprise a bacterial strain of the species *Blautia hydrogenotrophica*. The examples demonstrate that bacteria of this species are useful for treating or preventing an autoimmune or inflammatory disorder of the central nervous system, such as multiple sclerosis. The *Blautia* species are Gram-reaction-positive, non-motile bacteria that may be either coccoid or oval and all are obligate anaerobes that produce acetic acid as the major end product of glucose fermentation [20]. Blautia may be isolated from the human gut, although *B. producta* was isolated from a septicaemia sample. *Blautia hydrogenotrophica* (previously known as *Ruminococcus hydrogenotrophicus*) has been isolated from the guts of mammals, is strictly anaerobic, and metabolises H_2/CO_2 to acetate, which may be important for human nutrition. The type strain of *Blautia hydrogenotrophica* is S5a33 = JCM 14656. The GenBank accession number for the 16S rRNA gene sequence of *Blautia hydrogenotrophica* strain S5a36 is X95624.1 (disclosed herein as SEQ ID NO:1). This exemplary *Blautia hydrogenotrophica* strain is described in [20] and [21]. The S5a33 strain and the S5a36 strain correspond to two subclones of a strain isolated from a faecal sample of a healthy subject. They show identical morphology, physiology and metabolism and have identical 16S rRNA sequences. Thus, in some embodiments, the *Blautia hydrogenotrophica* for use in the invention has the 16S rRNA sequence of SEQ ID NO:1.

The *Blautia hydrogenotrophica* bacterium deposited under accession number DSM 14294 was tested in the examples and is also referred to herein as strain BH or Blautix. It is the preferred strain of the invention. Strain BH was deposited with the Deutsche Sammlung von Mikroorganismen [German Microorganism Collection] (Mascheroder Weg 1b, 38124 Braunschweig, Germany) under accession DSM 14294 as "S5a33" on 10th May 2001. The depositor was INRA Laboratoire de Microbiologie CR de Clermont-Ferrand/Theix 63122 Saint Genès Champanelle, France. Ownership of the deposits has passed to 4D Pharma Plc by way of assignment. 4D Pharma Plc has authorised, by way of an agreement, 4D Pharma Research Limited to refer to the deposited biological material in the application

and has given its unreserved and irrevocable consent to the deposited material being made available to the public.

Bacterial strains closely related to the strain tested in the examples are also expected to be effective for treating or preventing autoimmune or inflammatory disorders of the central nervous system. In certain embodiments, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to the 16s rRNA sequence of a bacterial strain of *Blautia hydrogenotrophica.* Preferably, the bacterial strain for use in the invention has a 16s rRNA sequence that is at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1.

Bacterial strains that are biotypes of the bacterium deposited under accession number DSM 14294 are also expected to be effective for treating or preventing autoimmune or inflammatory disorders of the central nervous system. A biotype is a closely related strain that has the same or very similar physiological and biochemical characteristics.

Strains that are biotypes of a bacterium deposited under accession number DSM 14294 and that are suitable for use in the invention may be identified by sequencing other nucleotide sequences for a bacterium deposited under accession number DSM 14294. For example, substantially the whole genome may be sequenced and a biotype strain for use in the invention may have at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity across at least 80% of its whole genome (e.g. across at least 85%, 90%, 95% or 99%, or across its whole genome). For example, in some embodiments, a biotype strain has at least 98% sequence identity across at least 98% of its genome or at least 99% sequence identity across 99% of its genome. Other suitable sequences for use in identifying biotype strains may include hsp60 or repetitive sequences such as BOX, ERIC, $(GTG)_5$, or REP or [22]. Biotype strains may have sequences with at least 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of a bacterium deposited under accession number DSM 14294. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited as DSM 14294 and comprises a 16S rRNA sequence that is at least 99% identical (e.g. at least 99.5% or at least 99.9% identical) to SEQ ID NO:1. In some embodiments, a biotype strain has a sequence with at least 97%, 98%, 99%, 99.5% or 99.9% sequence identity to the corresponding sequence of the *Blautia hydrogenotrophica* strain deposited as DSM 14294 and has the 16S rRNA sequence of SEQ ID NO1.

Alternatively, strains that are biotypes of a bacterium deposited under accession number DSM 14294and that are suitable for use in the invention may be identified by using the accession number DSM 14294 deposit and restriction fragment analysis and/or PCR analysis, for example by using fluorescent amplified fragment length polymorphism (FAFLP) and repetitive DNA element (rep)-PCR

fingerprinting, or protein profiling, or partial 16S or 23s rDNA sequencing. In preferred embodiments, such techniques may be used to identify other *Blautia hydrogenotrophica* strains.

In certain embodiments, strains that are biotypes of a bacterium deposited under accession number DSM 14294 and that are suitable for use in the invention are strains that provide the same pattern as a bacterium deposited under accession number DSM 14294 when analysed by amplified ribosomal DNA restriction analysis (ARDRA), for example when using Sau3AI restriction enzyme (for exemplary methods and guidance see, for example,[23]). Alternatively, biotype strains are identified as strains that have the same carbohydrate fermentation patterns as a bacterium deposited under accession number DSM 14294.

Other *Blautia hydrogenotrophica* strains that are useful in the compositions and methods of the invention, such as biotypes of a bacterium deposited under accession number DSM 14294, may be identified using any appropriate method or strategy, including the assays described in the examples. For instance, strains for use in the invention may be identified by culturing bacteria and administering to mice using an EAE model protocol, such as that used in the examples. In particular, bacterial strains that have similar growth patterns, metabolic type and/or surface antigens to a bacterium deposited under accession number DSM 14294 may be useful in the invention. A useful strain will have comparable microbiota modulatory activity to the DSM 14294 strain. In particular, a biotype strain will elicit comparable effects on autoimmune or inflammatory disorders of the central nervous system to the effects shown in the examples, which may be identified by using the culturing and administration protocols described in the examples.

A particularly preferred strain of the invention is the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294. This is the exemplary BH strain tested in the examples and shown to be effective for treating disease. Therefore, the invention provides a cell, such as an isolated cell, of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294, or a derivative thereof, for use in therapy, in particular for the diseases described herein.

A derivative of the strain deposited under accession number DSM 14294 may be a daughter strain (progeny) or a strain cultured (subcloned) from the original. A derivative of a strain of the invention may be modified, for example at the genetic level, without ablating the biological activity. In particular, a derivative strain of the invention is therapeutically active. A derivative strain will have comparable microbiota modulatory activity to the original DSM 14294 strain. In particular, a derivative strain will elicit comparable effects on autoimmune or inflammatory disorders of the central nervous system to the effects shown in the Examples, which may be identified by using the culturing and administration protocols described in the Examples. A derivative of the DSM 14294 strain will generally be a biotype of the DSM 14294 strain.

References to cells of the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294 encompass any cells that have the same safety and therapeutic efficacy characteristics as the strains deposited under accession number DSM 14294, and such cells are encompassed by the invention.

In preferred embodiments, the bacterial strains in the compositions of the invention are viable and capable of partially or totally colonising the intestine.

Therapeutic uses

The compositions of the inventor are for use in treating or preventing autoimmune or inflammatory disorders of the central nervous system. The examples demonstrate that the compositions of the invention achieve a reduction in the disease incidence and disease severity in a mouse model of CNS inflammation (the EAE model), and so they may be useful in the treatment or prevention of such conditions.

In preferred embodiments, the compositions of the invention are for use in treating or preventing a demyelinating autoimmune disease. The effects shown in the examples are particularly relevant for such diseases.

In preferred embodiments, the compositions of the invention are for use in treating or preventing an inflammatory demyelinating disease. The effects shown in the examples are particularly relevant for such diseases.

In particularly preferred embodiments, the compositions of the invention are for use in treating or preventing multiple sclerosis, as discussed in more detail below.

In certain embodiments, the disorder primarily affects the spine. In certain embodiments, the disorder primarily affects the spinal cord. In certain embodiments, the disorder primarily affects the brain.

In certain embodiments, treatment with a composition of the invention reduces inflammation in the spinal cord. In certain embodiments, treatment with a composition of the invention reduces inflammation in the brain. In certain embodiments, treatment with a composition of the invention reduces inflammation in the spinal cord and in the brain.

In preferred embodiments, the compositions is for use in treating or preventing a disease selected from the list consisting of: multiple sclerosis, neuromyelitis optica, anti-MOG autoimmune encephalomyelitis, chronic relapsing inflammatory optic neuritis, acute disseminated encephalomyelitis, acute hemorrhagic leukoencephalitis, balo concentric sclerosis, diffuse myelinoclastic sclerosis, Marburg multiple sclerosis, Tumefactive multiple sclerosis and solitary sclerosis. The effects shown in the examples are particularly relevant for such diseases, which are also

known as inflammatory demyelinating diseases (IDDs), or idiopathic inflammatory demyelinating diseases (IIDDs) or borderline forms of multiple sclerosis.

In certain embodiments, the compositions are for use in treating or preventing transverse myelitis, Bickerstaff brainstem encephalitis, Miller Fisher syndrome, CNS vasculitis, neurosarcoidosis, neuropsychiatric manifestations of systemic lupus erythematosus, tropical spastic paraparesis (TSP)/HTLV-I-associated myelopathy (HAM), or West Nile virus infection of the CNS.

In certain embodiments, the compositions of the invention are for use in a patient diagnosed with an infectious disease known to cause autoimmune or inflammatory disorders of the central nervous system, such as *Campylobacter jejuni* infection.

In certain embodiments, treatment with the compositions of the invention results in a reduction in disease incidence or disease severity. In certain embodiments, the compositions of the invention are for use in reducing disease incidence or disease severity. In certain embodiments, treatment with the compositions of the invention prevents a decline in motor function or results in improved motor function. In certain embodiments, the compositions of the invention are for use in preventing a decline in motor function or for use in improving motor function. In certain embodiments, treatment with the compositions of the invention prevents the development of paralysis.

In particularly preferred embodiments, the compositions of the invention are for use in treating or preventing multiple sclerosis. The examples demonstrate that the compositions of the invention achieve a reduction in the disease incidence and disease severity in a mouse model of multiple sclerosis (the EAE model), and so they may be useful in the treatment or prevention of multiple sclerosis. Multiple sclerosis is an inflammatory disorder and a demyelinating disease of the central nervous system associated with damage to the myelin sheaths of neurons, particularly in the brain and spinal column. Multiple sclerosis is a chronic disease, which is progressively incapacitating and which evolves in episodes. MS is usually found in older patients. Inflammation consisting of T cell and B cell infiltrates is usually found in the CNS and lesions of MS patients. The degree of lymphocyte infiltration is greater in the earlier phases of the disease as opposed to the later phases of the disease. CD8+ T cells are the predominant lymphocyte population with lower levels of CD4+ T cells and B cells. The compositions of the invention may be particularly effective for preventing or treating multiple sclerosis.

In certain embodiments, treatment with the compositions of the invention results in a reduction in MS incidence or MS severity. In certain embodiments, the compositions of the invention are for use in reducing relapse incidence or relapse severity. In certain embodiments, treatment with the compositions of the invention prevents a decline in motor function or results in improved motor function associated with MS. In certain embodiments, the compositions of the invention are for use in preventing a decline in motor function or for use in improving motor function in the treatment of MS.

In certain embodiments, treatment with the compositions of the invention prevents the development of paralysis in MS. In certain embodiments, the compositions of the invention are for use in preventing paralysis in the treatment of MS.

In certain embodiments the compositions of the invention are for use in preventing multiple sclerosis in a patient that has been identified as at risk of multiple sclerosis, or that has been diagnosed with early-stage multiple sclerosis or "relapsing-remitting" multiple sclerosis. The compositions of the invention may be useful for preventing the development of MS. The compositions of the invention may be useful for preventing the progression of MS. In certain embodiments, the compositions of the invention are for use in a patient identified as having a genetic predisposition to MS, such as major histocompatibility complex (MHC) class II phenotype, human leukocyte antigen (HLA)-DR2 or HLA-DR4.

The compositions of the invention may be useful for managing or alleviating multiple sclerosis. The compositions of the invention may be particularly useful for reducing symptoms associated with multiple sclerosis. Treatment or prevention of multiple sclerosis may refer to, for example, an alleviation of the severity of symptoms or a reduction in the frequency of exacerbations or the range of triggers that are a problem for the patient. In certain embodiments, the compositions of the invention slow or stop progression of the disease.

In certain embodiments, the compositions of the invention are for use in treating relapsing-remitting MS. In alternative embodiments, the compositions of the invention are for use in treating progressive MS, such as secondary progressive MS (SPMS), which develops over time following diagnosis of RRMS, primary progressive MS (PPMS) which exhibits gradual continuous neurologic deterioration and progressive relapsing MS (PRMS), which is similar to PPMS but with overlapping relapses.

In certain embodiments, the compositions of the invention are for use in treating one or more of symptoms of MS selected from the group consisting of: fatigue, vision problems, numbness, tingling, muscle spasms, muscle stiffness, muscle weakness, mobility problems, pain, problems with thinking, learning and planning, depression and anxiety, sexual problems, bladder problems, bowel problems, speech and swallowing difficulties.

In certain embodiments, the compositions of the invention are for use in combination with a secondary active agent. In certain embodiments, the compositions of the invention are for use in combination with β-interferon 1a or 1b or glatiramer acetate. Other secondary agents include other interferons, dimethyl fumarate, teriflunomide, fingolimod, mitoxantrone, humanized monoclonal antibodies (such as natalizumab, ofatumumab, ocrelizumab, alemtuzumab, daclizumab), stem cells, DNA vaccines, nanoparticles and altered peptide ligands. The compositions of the invention may improve the patient's response to the secondary active agent.

Histone deacetylase inhibitors such as butyrate have also been proposed for use in the treatment of multiple sclerosis [24].

In certain embodiments, the composition of the invention comprising *Blautia hydrogenotrophica* inhibits neuro-inflammation. In certain embodiments, the composition of the invention comprising *Blautia hydrogenotrophica* increases the levels of IL-1RA (an inhibitor of the pro-inflammatory IL-1β). In certain embodiments, the composition of the invention comprising *Blautia hydrogenotrophica* decreases the levels pro-inflammatory IL-1β and/or TNFα. In certain embodiments, the composition of the invention comprising *Blautia hydrogenotrophica* increases IL-4 expression, which increases the levels of IL-1RA. In certain embodiments, the composition of the invention comprising *Blautia hydrogenotrophica* inhibits nuclear factor κB (NF- κB) activation. Accordingly, the composition of the invention comprising *Blautia hydrogenotrophica* may modulate the expression of early immune inflammatory response genes, including IL-1B, TNFα, IL-2, IL-6, IL-8, IL-12, inducible nitric acid synthase, cyclooxygenase-2, intercellular adhesion molecule-1, T cell receptor-α and MHC class II molecules.

The compositions of the inventor are for use in treating or preventing autoimmune or inflammatory disorders of the central nervous system. Thus, in certain embodiments, the disorder to be treated by the composition of the invention is not an autism spectrum disorder (ASDs); child developmental disorder; obsessive compulsive disorder (OCD); major depressive disorder; depression; seasonal affective disorder; an anxiety disorder; chronic fatigue syndrome (myalgic encephalomyelitis); stress disorder; post-traumatic stress disorder; a schizophrenia spectrum disorder; schizophrenia; bipolar disorder; psychosis; mood disorder; dementia; Alzheimer's; Parkinson's disease; chronic pain, motor neuron disease; Huntington's disease; Guillain-Barre syndrome or meningitis.

Modes of administration

Preferably, the compositions of the invention are to be administered to the gastrointestinal tract in order to enable delivery to and / or partial or total colonisation of the intestine with the bacterial strain of the invention. Generally, the compositions of the invention are administered orally, but they may be administered rectally, intranasally, or via buccal or sublingual routes.

In certain embodiments, the compositions of the invention may be administered as a foam, as a spray or a gel.

In certain embodiments, the compositions of the invention may be administered as a suppository, such as a rectal suppository, for example in the form of a theobroma oil (cocoa butter), synthetic hard fat (e.g. suppocire, witepsol), glycero-gelatin, polyethylene glycol, or soap glycerin composition.

In certain embodiments, the composition of the invention is administered to the gastrointestinal tract via a tube, such as a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J tube),

percutaneous endoscopic gastrostomy (PEG), or a port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

The compositions of the invention may be administered once, or they may be administered sequentially as part of a treatment regimen. In certain embodiments, the compositions of the invention are to be administered daily. The examples demonstrate that administration provides successful colonisation and clinical benefits in treatment of autoimmune or inflammatory disorders of the central nervous system.

In certain embodiments, the compositions of the invention are administered regularly, such as daily, every two days, or weekly, for an extended period of time, such as for at least one week, two weeks, one month, two months, six months, or one year. The examples demonstrate that BH administration may not result in permanent colonisation of the intestines, so regular administration for extended periods of time may provide greater therapeutic benefits.

In some embodiments the compositions of the invention are administered for 7 days, 14 days, 16 days, 21 days or 28 days or no more than 7 days, 14 days, 16 days, 21 days or 28 days. For example, in some embodiments the compositions of the invention are administered for 16 days.

In certain embodiments of the invention, treatment according to the invention is accompanied by assessment of the patient's gut microbiota. Treatment may be repeated if delivery of and / or partial or total colonisation with the strain of the invention is not achieved such that efficacy is not observed, or treatment may be ceased if delivery and / or partial or total colonisation is successful and efficacy is observed.

In certain embodiments, the composition of the invention may be administered to a pregnant animal, for example a mammal such as a human in order to prevent autoimmune or inflammatory disorders of the central nervous system developing in her child *in utero* and / or after it is born.

The compositions of the invention may be administered to a patient that has been diagnosed with an autoimmune or inflammatory disorder of the central nervous system, or that has been identified as being at risk of such a disorder. The compositions may also be administered as a prophylactic measure to prevent the development of disease in a healthy patient.

The compositions of the invention may be administered to a patient that has been identified as having an abnormal gut microbiota. For example, the patient may have reduced or absent colonisation by *Blautia*, and in particular *Blautia hydrogenotrophica.*

The compositions of the invention may be administered as a food product, such as a nutritional supplement.

Generally, the compositions of the invention are for the treatment of humans, although they may be used to treat animals including monogastric mammals such as poultry, pigs, cats, dogs, horses or rabbits. The compositions of the invention may be useful for enhancing the growth and performance of animals. If administered to animals, oral gavage may be used.

In some embodiments, the subject to whom the composition is to be administered is an adult human. In some embodiments, the subject to whom the composition is to be administered is an infant human.

Compositions

Generally, the composition of the invention comprises bacteria. In preferred embodiments of the invention, the composition is formulated in freeze-dried form. For example, the composition of the invention may comprise granules or gelatin capsules, for example hard gelatin capsules, comprising a bacterial strain of the invention.

Preferably, the composition of the invention comprises lyophilised bacteria. Lyophilisation of bacteria is a well-established procedure and relevant guidance is available in, for example, references [25-27]. Lyophilisate compositions may be particularly effective. In preferred embodiments, the compositions of the invention comprises lyophilised bacteria and is for the treatment of MS.

Alternatively, the composition of the invention may comprise a live, active bacterial culture. In some embodiments, the bacterial strain in the composition of the invention has not been inactivated, for example, has not been heat-inactivated. In some embodiments, the bacterial strain in the composition of the invention has not been killed, for example, has not been heat-killed. In some embodiments, the bacterial strain in the composition of the invention has not been attenuated, for example, has not been heat-attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention has not been killed, inactivated and/or attenuated. For example, in some embodiments, the bacterial strain in the composition of the invention is live. For example, in some embodiments, the bacterial strain in the composition of the invention is viable. For example, in some embodiments, the bacterial strain in the composition of the invention is capable of partially or totally colonising the intestine. For example, in some embodiments, the bacterial strain in the composition of the invention is viable and capable of partially or totally colonising the intestine.

In some embodiments, the composition comprises a mixture of live bacterial strains and bacterial strains that have been killed.

In preferred embodiments, the composition of the invention is encapsulated to enable delivery of the bacterial strain to the intestine. Encapsulation protects the composition from degradation until delivery at the target location through, for example, rupturing with chemical or physical stimuli such as pressure, enzymatic activity, or physical disintegration, which may be triggered by changes in pH. Any appropriate encapsulation method may be used. Exemplary encapsulation techniques include

entrapment within a porous matrix, attachment or adsorption on solid carrier surfaces, self-aggregation by flocculation or with cross-linking agents, and mechanical containment behind a microporous membrane or a microcapsule. Guidance on encapsulation that may be useful for preparing compositions of the invention is available in, for example, references [28-29].

The composition may be administered orally and may be in the form of a tablet, capsule or powder. Encapsulated products are preferred because *Blautia* are anaerobes. Other ingredients (such as vitamin C, for example), may be included as oxygen scavengers and prebiotic substrates to improve the delivery and / or partial or total colonisation and survival *in vivo*. Alternatively, the probiotic composition of the invention may be administered orally as a food or nutritional product, such as milk or whey based fermented dairy product, or as a pharmaceutical product.

The composition may be formulated as a probiotic.

A composition of the invention includes a therapeutically effective amount of a bacterial strain of the invention. A therapeutically effective amount of a bacterial strain is sufficient to exert a beneficial effect upon a patient. A therapeutically effective amount of a bacterial strain may be sufficient to result in delivery to and / or partial or total colonisation of the patient's intestine.

A suitable daily dose of the bacteria, for example for an adult human, may be from about 1×10^3 to about 1×10^{11} colony forming units (CFU); for example, from about 1×10^7 to about 1×10^{10} CFU; in another example from about 1×10^6 to about 1×10^{10} CFU; in another example from about 1×10^7 to about 1×10^{11} CFU; in another example from about 1×10^8 to about 1×10^{10} CFU; in another example from about 1×10^8 to about 1×10^{11} CFU.

In certain embodiments, the dose of the bacteria is at least 10^9 cells per day, such as at least 10^{10}, at least 10^{11}, or at least 10^{12} cells per day.

In certain embodiments, the composition contains the bacterial strain in an amount of from about 1×10^6 to about 1×10^{11} CFU/g, respect to the weight of the composition; for example, from about 1×10^8 to about 1×10^{10} CFU/g. The dose may be, for example, 1 g, 3g, 5g, and 10g. In some embodiments, the dose may be 1g or less, for example, from about 0.5g to about 1g, for example, about 0.5g, 0.6g, 0.75g, 0.8g, 0.9g or 1g.

Typically, a probiotic, such as the composition of the invention, is optionally combined with at least one suitable prebiotic compound. A prebiotic compound is usually a non-digestible carbohydrate such as an oligo- or polysaccharide, or a sugar alcohol, which is not degraded or absorbed in the upper digestive tract. Known prebiotics include commercial products such as inulin and transgalacto-oligosaccharides.

In certain embodiments, the probiotic composition of the present invention includes a prebiotic compound in an amount of from about 1 to about 30% by weight, respect to the total weight composition, (e.g. from 5 to 20% by weight). Carbohydrates may be selected from the group consisting of: fructo- oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), beta-glucans, arable gum modified and resistant starches, polydextrose, D-tagatose, acacia fibers, carob, oats, and citrus fibers. In one aspect, the prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown herein below as FOSs-c.c); said FOSs-c.c. are not digestible carbohydrates, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

The compositions of the invention may comprise pharmaceutically acceptable excipients or carriers. Examples of such suitable excipients may be found in the reference [30]. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for example, in reference [31]. Examples of suitable carriers include lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol and the like. Examples of suitable diluents include ethanol, glycerol and water. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as, or in addition to, the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s). Examples of suitable binders include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweeteners, natural and synthetic gums, such as acacia, tragacanth or sodium alginate, carboxymethyl cellulose and polyethylene glycol. Examples of suitable lubricants include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid, cysteine and esters of p-hydroxybenzoic acid, for example, in some embodiments the preservative is selected from sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used. A further example of a suitable carrier is saccharose. A further example of a preservative is cysteine.

The compositions of the invention may be formulated as a food product. For example, a food product may provide nutritional benefit in addition to the therapeutic effect of the invention, such as in a nutritional supplement. Similarly, a food product may be formulated to enhance the taste of the composition of the invention or to make the composition more attractive to consume by being more similar to a common food item, rather than to a pharmaceutical composition. In certain embodiments, the composition of the invention is formulated as a milk-based product. The term "milk-based product" means any liquid or semi-solid milk- or whey- based product having a varying fat content. The milk-

based product can be, e.g., cow's milk, goat's milk, sheep's milk, skimmed milk, whole milk, milk recombined from powdered milk and whey without any processing, or a processed product, such as yoghurt, curdled milk, curd, sour milk, sour whole milk, butter milk and other sour milk products. Another important group includes milk beverages, such as whey beverages, fermented milks, condensed milks, infant or baby milks; flavoured milks, ice cream; milk-containing food such as sweets.

In some embodiments, the compositions of the invention comprise one or more bacterial strains of the genus *Blautia* and do not contain bacteria from any other genus, or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus.

In some embodiments, the compositions of the invention comprise one or more bacterial strains of the species *Blautia hydrogenotrophica* and do not contain bacteria from any other species, or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species.

In some embodiments, the composition of the invention comprises a single strain of *Blautia hydrogenotrophica,* preferably strain BH, and does not contain bacteria from any other strains, or comprise only *de minimis* or biologically irrelevant amounts of bacteria from another strain.

In certain embodiments, the compositions of the invention contain a single bacterial strain or species and do not contain any other bacterial strains or species. Such compositions may comprise only *de minimis* or biologically irrelevant amounts of other bacterial strains or species. Such compositions may be a culture that is substantially free from other species of organism. In some embodiments, such compositions may be a lyophilisate that is substantially free from other species of organism.

In certain embodiments, the compositions of the invention comprise one or more bacterial strains of the genus *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial genus, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another genus. In certain embodiments, the compositions of the invention comprise a single species of *Blautia,* for example, a *Blautia hydrogenotrophica,* and do not contain any other bacterial species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another species. In certain embodiments, the compositions of the invention comprise a single strain of *Blautia,* for example, of *Blautia hydrogenotrophica,* and do not contain any other bacterial strains or species, or which comprise only *de minimis* or biologically irrelevant amounts of bacteria from another strain or species.

In some embodiments, the compositions of the invention comprise more than one bacterial strain or species. For example, in some embodiments, the compositions of the invention comprise more than one strain from within the same species (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 or 45 strains), and, optionally, do not contain bacteria from any other species. In some

embodiments, the compositions of the invention comprise less than 50 strains from within the same species (e.g. less than 45, 40, 35, 30, 25, 20, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 strains), and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise 1-40, 1-30, 1-20, 1-19, 1-18, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 strains from within the same species and, optionally, do not contain bacteria from any other species. In some embodiments, the compositions of the invention comprise more than one species from within the same genus (e.g. more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, 23, 25, 30, 35 or 40 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise less than 50 species from within the same genus (e.g. less than 50, 45, 40, 35, 30, 25, 20, 15, 12, 10, 8, 7, 6, 5, 4 or 3 species), and, optionally, do not contain bacteria from any other genus. In some embodiments, the compositions of the invention comprise 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-50, 2-40, 2-30, 2-20, 2-15, 2-10, 2-5, 6-30, 6-15, 16-25, or 31-50 species from within the same genus and, optionally, do not contain bacteria from any other genus. The invention comprises any combination of the foregoing.

In some embodiments, the composition comprises a microbial consortium. For example, in some embodiments, the composition comprises the *Blautia hydrogenotrophica* bacterial strain as part of a microbial consortium. For example, in some embodiments, the *Blautia hydrogenotrophica* bacterial strain is present in combination with one or more (e.g. at least 2, 3, 4, 5, 10, 15 or 20) other bacterial strains from other genera with which it can live symbiotically *in vivo* in the intestine. For example, in some embodiments, the composition comprises a bacterial strain of *Blautia hydrogenotrophica* in combination with a bacterial strain from a different genus. In some embodiments, the microbial consortium comprises two or more bacterial strains obtained from a faeces sample of a single organism, e.g. a human. In some embodiments, the microbial consortium is not found together in nature. For example, in some embodiments, the microbial consortium comprises bacterial strains obtained from faeces samples of at least two different organisms. In some embodiments, the two different organisms are from the same species, e.g. two different humans. In some embodiments, the two different organisms are an infant human and an adult human. In some embodiments, the two different organisms are a human and a non-human mammal.

In some embodiments, the composition of the invention additionally comprises a bacterial strain that has the same safety and therapeutic efficacy characteristics as the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294, but which is not the *Blautia hydrogenotrophica* strain deposited under accession number DSM 14294, or which is not a *Blautia hydrogenotrophica* or which is not a *Blautia*.

In some embodiments in which the composition of the invention comprises more than one bacterial strain, species or genus, the individual bacterial strains, species or genera may be for separate, simultaneous or sequential administration. For example, the composition may comprise all of the more than one bacterial strain, species or genera, or the bacterial strains, species or genera may be stored separately and be administered separately, simultaneously or sequentially. In some embodiments, the more than one bacterial strains, species or genera are stored separately but are mixed together prior to use.

In some embodiments, the bacterial strain for use in the invention is obtained from human adult faeces. In some embodiments in which the composition of the invention comprises more than one bacterial strain, all of the bacterial strains are obtained from human adult faeces or if other bacterial strains are present they are present only in *de minimis* amounts. The bacteria may have been cultured subsequent to being obtained from the human adult faeces and being used in a composition of the invention.

In some embodiments, the one or more *Blautia* bacterial strains is/are the only therapeutically active agent(s) in a composition of the invention. In some embodiments, the bacterial strain(s) in the composition is/are the only therapeutically active agent(s) in a composition of the invention.

The compositions for use in accordance with the invention may or may not require marketing approval.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised. In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is spray dried. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is live. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is capable of partially or totally colonising the intestine. In certain embodiments, the invention provides the above pharmaceutical composition, wherein the bacterial strain is lyophilised or spray dried and wherein it is viable and capable of partially or totally colonising the intestine.

In some cases, the lyophilised or spray dried bacterial strain is reconstituted prior to administration. In some cases, the reconstitution is by use of a diluent described herein.

The compositions of the invention can comprise pharmaceutically acceptable excipients, diluents or carriers.

In certain embodiments, the invention provides a pharmaceutical composition comprising: a bacterial strain of the invention; and a pharmaceutically acceptable excipient, carrier or diluent; wherein the bacterial strain is in an amount sufficient to treat a disorder when administered to a subject in need

thereof; and wherein the disorder is an autoimmune or inflammatory disorder of the central nervous system.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the amount of the bacterial strain is from about 1×10^3 to about 1×10^{11} colony forming units per gram with respect to a weight of the composition.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered at a dose of 1 g, 3 g, 5 g or 10 g.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein the composition is administered by a method selected from the group consisting of oral, rectal, subcutaneous, nasal, buccal, and sublingual.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a carrier selected from the group consisting of lactose, starch, glucose, methyl cellulose, magnesium stearate, mannitol and sorbitol.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a diluent selected from the group consisting of ethanol, glycerol and water.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising an excipient selected from the group consisting of starch, gelatin, glucose, anhydrous lactose, free-flow lactose, beta-lactose, corn sweetener, acacia, tragacanth, sodium alginate, carboxymethyl cellulose, polyethylene glycol, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate and sodium chloride.

In certain embodiments, the invention provides the above pharmaceutical composition, further comprising at least one of a preservative, an antioxidant and a stabilizer.

In certain embodiments, the invention provides the above pharmaceutical composition, comprising a preservative selected from the group consisting of sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein said bacterial strain is lyophilised.

In certain embodiments, the invention provides the above pharmaceutical composition, wherein when the composition is stored in a sealed container at about 4◦C or about 25◦C and the container is placed in an atmosphere having 50% relative humidity, at least 80% of the bacterial strain as measured in colony forming units, remains after a period of at least about: 1 month, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years.

In some embodiments, the composition of the invention is provided in a sealed container comprising a composition as described herein. In some embodiments, the sealed container is a sachet or bottle. In some embodiments, the composition of the invention is provided in a syringe comprising a composition as described herein.

The composition of the present invention may, in some embodiments, be provided as a pharmaceutical formulation. For example, the composition may be provided as a tablet or capsule. In some embodiments, the composition may be provided in the form of one tablet or capsule or more than one tablet or capsule, for example, 1, 2, 3, 4, 5 or more tablets or capsules. In some embodiments, the capsule is a gelatine capsule ("gel-cap").

In some embodiments, the compositions of the invention are administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

Pharmaceutical formulations suitable for oral administration include solid plugs, solid microparticulates, semi-solid and liquid (including multiple phases or dispersed systems) such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids (e.g. aqueous solutions), emulsions or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

In some embodiments the pharmaceutical formulation is an enteric formulation, i.e. a gastro-resistant formulation (for example, resistant to gastric pH) that is suitable for delivery of the composition of the invention to the intestine by oral administration. Enteric formulations may be particularly useful when the bacteria or another component of the composition is acid-sensitive, e.g. prone to degradation under gastric conditions.

In some embodiments, the enteric formulation comprises an enteric coating. In some embodiments, the formulation is an enteric-coated dosage form. For example, the formulation may be an enteric-coated tablet or an enteric-coated capsule, or the like. The enteric coating may be a conventional enteric coating, for example, a conventional coating for a tablet, capsule, or the like for oral delivery. The formulation may comprise a film coating, for example, a thin film layer of an enteric polymer, e.g. an acid-insoluble polymer.

In some embodiments, the enteric formulation is intrinsically enteric, for example, gastro-resistant without the need for an enteric coating. Thus, in some embodiments, the formulation is an enteric formulation that does not comprise an enteric coating. In some embodiments, the formulation is a capsule made from a thermogelling material. In some embodiments, the thermogelling material is a cellulosic material, such as methylcellulose, hydroxymethylcellulose or hydroxypropylmethylcellulose (HPMC). In some embodiments, the capsule comprises a shell that

does not contain any film forming polymer. In some embodiments, the capsule comprises a shell and the shell comprises hydroxypropylmethylcellulose and does not comprise any film forming polymer (e.g. see [32]). In some embodiments, the formulation is an intrinsically enteric capsule (for example, Vcaps® from Capsugel).

In some embodiments, the formulation is a soft capsule. Soft capsules are capsules which may, owing to additions of softeners, such as, for example, glycerol, sorbitol, maltitol and polyethylene glycols, present in the capsule shell, have a certain elasticity and softness. Soft capsules can be produced, for example, on the basis of gelatine or starch. Gelatine-based soft capsules are commercially available from various suppliers. Depending on the method of administration, such as, for example, orally or rectally, soft capsules can have various shapes, they can be, for example, round, oval, oblong or torpedo-shaped. Soft capsules can be produced by conventional processes, such as, for example, by the Scherer process, the Accogel process or the droplet or blowing process.

Culturing methods

The bacterial strains for use in the present invention can be cultured using standard microbiology techniques as detailed in, for example, references [33-35].

The solid or liquid medium used for culture may for example be YCFA agar or YCFA medium. YCFA medium may include (per 100ml, approximate values): Casitone (1.0 g), yeast extract (0.25 g), $NaHCO_3$ (0.4 g), cysteine (0.1 g), K_2HPO_4 (0.045 g), KH_2PO_4 (0.045 g), NaCl (0.09 g), $(NH_4)_2SO_4$ (0.09 g), $MgSO_4 \cdot 7H_2O$ (0.009 g), $CaCl_2$ (0.009 g), resazurin (0.1 mg), hemin (1 mg), biotin (1 μg), cobalamin (1 μg), *p*-aminobenzoic acid (3 μg), folic acid (5 μg), and pyridoxamine (15 μg).

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references [36-43], *etc.*

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

References to a percentage sequence identity between two nucleotide sequences means that, when aligned, that percentage of nucleotides are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in

the art, for example those described in section 7.7.18 of ref. [44]. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. [45].

Unless specifically stated, a process or method comprising numerous steps may comprise additional steps at the beginning or end of the method, or may comprise additional intervening steps. Also, steps may be combined, omitted or performed in an alternative order, if appropriate.

Various embodiments of the invention are described herein. It will be appreciated that the features specified in each embodiment may be combined with other specified features, to provide further embodiments. In particular, embodiments highlighted herein as being suitable, typical or preferred may be combined with each other (except when they are mutually exclusive).

MODES FOR CARRYING OUT THE INVENTION

Example 1

Experimental Autoimmune Encephalomyelitis (EAE) is a mouse model of CNS inflammation that mirrors many aspects of the human disease MS and EAE is the most commonly used experimental model for human MS. EAE is also used more generally as a model for CNS-specific autoimmune disorders [46] and for other specific conditions, including acute disseminated encephalomyelitis. EAE is induced using immunisation with myelin peptides and adjuvants to elicit an immune and inflammatory response that closely corresponds to the mechanisms underlying many autoimmune and inflammatory disorders of the CNS, and in particular MS. Many therapies showing efficacy in EAE have also shown efficacy in treatment of MS in human patients [46]. Most importantly, EAE reproduces key features of MS, including inflammation, demyelination, axonal loss and gliosis. The effects of demyelination are mainly restricted to the spinal cord in EAE, with little alteration of the brain stem and the cerebellum. In EAE the CD4+ T cells are the dominant cell population found in the CNS.

Methodology

Blautia hydrogenotrophica ("Blautix", strain deposited under accession number DSM 14294) was used as a freeze-dried powder and reconstituted as required.

Adult female C57BL/6J mice were randomly allocated to experimental groups and allowed to acclimatise for one week. All Groups were n = 12.

On Day 0 and Day 7, animals in Groups 2, 3, 8 and 9 were administered with an emulsion containing MOG35-55 and complete Freund's adjuvant (CFA) supplemented with Mycobacterium Tuberculosis H37Ra by subcutaneous injections under gas (isoflurane) anaesthesia. On Day 0, two subcutaneous

injections were performed in the flanks; one in each of the lower quadrant of the back. On Day 7, two subcutaneous injections were performed in the flanks, one in each of the upper quadrant of the back.

On Day 0 and Day 2, animals in Groups 2, 3, 8 and 9 were administered with pertussis toxin (PTx) in phosphate buffered saline (PBS) by intra-peritoneal injections. On Day 0, PTx administration was performed after MOG injections.

Treatments with Blautix or controls were administered from Day -14 according to the following schedule:

Groups		Dose	Route	Regimen	Disease Induction
1	Control	n/a	n/a	n/a	Day O: PBS, once, SC Day 0: PBS, once, IP Day 2: PBS, once, IP Day 7: PBS, once, SC
2	Vehicle (PBS)	n/a	PO	SID: Day -14-End	Day 0: MOG/CFA, once, SC Day 0: PTx, once, IP Day 2: PTx, once, IP Day 7: MOG/CFA, once, SC
3	Reference	TBC	SC	SID: Day -14-Day -1 (vehicle) SID: Day 0-End (reference)	
8	Blautix	Dose 1	PO	SID: Day -14-End	
9	Vehicle for Blautix	n/a	PO	SID: Day -14-End	

n/a: not applicable, SID: once per day, PO: oral administration (gavage), SC: subcutaneous injection, IP: intra-peritoneal injection, MOG: myelin oligodendrocyte glycoprotein, CFA: complete Freund's adjuvant, PTx: pertussis toxin, PBS: phosphate-buffered saline

Treatments were administered within 15 minutes of their preparation.

Group 3 mice were treated with the Reference dexamethasone as a positive control. For Group 3, a dose of 1 mg/kg (5 ml/kg) was used with mice being treated from Day -14 - Day -1 via the PO route with vehicle (PBS) only, 5 times a week (2 Days on, 1 Day off, 3 Days on, 1 Day off); and from Day 0 - End via the SC route with vehicle and Dexamethasone, 5 times a week (2 Days on, 1 Day off, 3 Days on, 1 Day off).

Blautix was administered at a dose of 2×10^8; 100µl/mouse.

From Day -14, animals were weighed three times per week. From Day 0 until the end of the experiment, animals were weighed daily. From Day 0 until the end of the experiment, animals were scored daily for clinical signs of EAE, including paresis and paralysis of the tail and/or limbs. On Day -14 and Day 35, blood samples were collected and processed to isolate serum. Day -14 and Day 35 samples were collected from a caudal (tail) vein in restrained animals. Samples were stored at -20°C until further optional analysis of anti-MOG antibodies by ELISA. On Day 35, animals were culled; brains and spinal cords were dissected out, one brain hemisphere and the spinal cord were transferred in tissue fixative then embedded in paraffin and stored in blocks until optional histopathology analysis. One

brain hemisphere was dissected out and was snap-frozen then stored at -80°C. On Day 35, spleens were dissected out, weighed and processed to cell suspension. One aliquot per animal was used for cell proliferation assays. One aliquot per animal was snap-frozen then stored at -80°C.

On Day 35, the full caecum (and its content) was snap-frozen in liquid nitrogen, and stored at -80°C. In addition, two centimetres of each of the following was frozen in RNAlater (>10 volumes), overnight at 4°C then stored at -80°C: ileum upstream of caecum, ascending colon, transverse colon and descending colon.

On Day -1 (baseline) and Day 34, faecal pellets were collected from each animal, immediately snap-frozen and stored at -80°C.

Non-specific clinical observations

From Day -14 (corresponding to the first treatment administration) until the end of the experiment, animals were checked daily for non-specific clinical signs to include abnormal posture (hunched), abnormal coat condition (piloerection) and abnormal activity levels (reduced or increased activity). Animals showing abnormal breathing were culled immediately. Animals were culled prior to the scheduled end of the study if non-specific clinical signs were judged too severe.

Scoring of clinical signs

Exemplary suggested disease activity score observations:

0 - No obvious changes in motor function compared to non-immunized mice.

0.5 - Tip of tail is limp.

1.0 - Limp tail.

1.5 - Limp tail and hind leg inhibition.

2.0 - Limp tail and weakness of hind legs.

OR - There are obvious signs of head tilting when the walk is observed. The balance is poor.

2.5 - Limp tail and dragging of hind legs.

OR - There is a strong head tilt that causes the mouse to occasionally fall over.

3.0 - Limp tail and complete paralysis of hind legs.

3.5 - Limp tail and complete paralysis of hind legs.

In addition to: Mouse is moving around the cage, but when placed on its side, is unable to right itself.

Hind legs are together on one side of body.

4.0 - Limp tail, complete hind leg and partial front leg paralysis.

Mouse is minimally moving around the cage but appears alert and feeding

4.5 - Complete hind and partial front leg paralysis, no movement around the cage. Mouse is immediately euthanized and removed from cage.

5.0 Mouse is euthanized due to severe paralysis.

When an animal has equal or greater disease activity score of 1, it may be considered to have a positive disease incidence score.

Animals with scores judged too severe were culled prior to the scheduled end of the experiment. Mice with a score of (5), corresponding to a moribund state, on any occasion were culled immediately. Mice with a score of (4), corresponding to a paralysis affecting both hind limbs and a front limb, on two consecutive occasions were culled. Mice with a score of (3), corresponding to a paralysis affecting both hind limb, on four consecutive occasions were culled. 2 mice in Group 2 were terminated due to EAE scores before the end of the experiment. 1 mouse in each of Groups 3 and 8 was terminated due to clinical observations before the end of the experiment. All mice in Groups 1 and 9 were terminated at the end of the experiment.

Histopathology

Sections of whole brains and longitudinal and cross-sections of spinal cords were stained with haematoxylin and eosin. Sections were evaluated in blinded fashion without knowledge of the experimental protocol.

The following scoring system was used:

Grade 0 - Histologically normal.

Grade 1 - Few discrete perivascular cuffs with no significant demyelination.

Grade 2 - Numerous discrete small to medium perivascular cuffs affecting parenchyma and meninges. May be focal demyelination of individual axons associated with mild extension of cuff to surrounding parenchyma.

Grade 3 - Numerous medium to large perivascular cuffs may coalesce and extend significantly into parenchyma. Meninges also involved. May be demyelination of axonal groups associated with extension into parenchyma.

Results

Clinical scores

Figures 1a and 2a show the results of the study at day 30. Figures 1b and 2b show the results of the study at day 35. Group 9 (Blautix-vehicle) shows that disease induction was successful and the model replicates some of the clinical features of MS with increased clinical scores relative to Group 1

(control). Group 3 (Dexamethasone) is a positive control showing successful amelioration of clinical signs.

A two-way ANOVA with Dunnet's *post-hoc* test to compare all treatment groups vs. vehicle (PBS; Group 2) revealed a significant effect of time and treatment and interaction between time and treatment (all $p < 0.0001$). Area under the curve (AUC) analysis revealed a significant effect of dexamethasone vs. vehicle-PBS control ($p < 0.001$), as assessed by a two-tailed unpaired two-sample Student's t-test. Administration of the bacterial composition of the invention (Group 8 – Blautix) provided a clear reduction in clinical scores relative to Group 9 and also Group 2 (PBS negative control), demonstrating a systemic effect of the treatment and successful treatment of the symptoms and clinical manifestations of EAE. These data indicate that Blautix may be useful for treating or preventing multiple sclerosis and other autoimmune or inflammatory disorders of the central nervous system.

Histopathology

Histopathological analysis revealed changes that are expected for this model in spinal cord and brain.

Spinal cord. Group 1 showed no pathology and Group 2 showed the most severe pathology in the highest proportion of animals of any group, as expected. Group 3 showed very limited pathology in only 2 animals. Groups 8 and 9 were more homogenous, showing less severe pathology than Group 2 affecting relatively fewer animals per group. Data was analysed using Mann-Whitney test to compare each treatment group to vehicle (PBS) group. This analysis revealed significantly decreased pathology in the dexamethasone and Blautix groups compared to vehicle (PBS) group ($p < 0.001$ and $p < 0.05$, respectively; Figure 3).

Figure 4 shows representative pictures of spinal cord sections stained with haematoxylin and eosin. **A.** Mouse 1.12, spinal cord x10. There are no histological abnormalities. **B.** and **C.** Mouse 2.7, spinal cord x10 and x20. Severe diffuse inflammation and demyelination of peripheral white matter with focal perivascular cuffing. **D.** Mouse 3.3, spinal cord x20. Discrete focus inflammation in peripheral white matter. **H.** Mouse 8.3, spinal cord, x20. Marked demyelination with spheroids and diffuse inflammation. **I.** Mouse 9.3, spinal cord, x10. Diffuse inflammation, demyelination and spheroid formation.

Brain. Group 1 animals showed no histological changes and there was a negligible change in group 3 with only one animal affected and very low mean pathology score. Although there were similar mean severity scores in groups 2, 8 and 9, there were fewer animals affected in groups 8 and 9 compared with group 2. There were some correlations with the spinal cord data (i.e. group 1 normal and group 3 limited pathology), but also some differences (i.e. group 2 has the most severe cord pathology, but not for brain). This is not unexpected for this model, in which cord pathology is often more consistent within groups than that occurring in the brain. Mann-Whitney test revealed significantly lower brain

pathology score in the dexamethasone group compared to vehicle (PBS) group ($p < 0.05$), as shown in Figure 3.

Figure 5 shows representative pictures of brain sections stained with haematoxylin and eosin. **A.** Mouse 1.1 x10, normal cerebellum. **B.** Mouse 2.7 x10, score 3. Perivascular cuffs and coalescing granulomatous inflammation cerebellar white matter. **H.** Mouse 8.5 x10, score 2. Discrete perivascular cuffs with milder diffuse inflammation cerebellar white matter. **I.** Mouse 9.1 x10, score 3. Multiple perivascular cuffs with diffuse inflammation cerebellar white matter.

To conclude, Blautix significantly improved spinal cord pathology compared to vehicle (PBS). Blautix also statistically significantly reduced inflammation in the spinal cord. There was also a strong trend showing a positive effect in the brain.

Anti-MOG antibody analysis

On Day -14 and at the end of the experiment, blood samples were collected and processed to isolate serum. On Day -14 and Day 35, blood samples were collected in restrained animals from a caudal (tail) vein (no more than 10% of the total blood volume). The serum samples were stored at -20°C until further analysis. Baseline serum samples and terminal serum samples from all animals were analysed for anti-peptide (MOG) total IgG antibody by ELISA with the coating antigen for each group being the peptide used for immunisation (MOG).

One-Way ANOVA followed by Dunnett's *post*-test for multiple comparison was used to determine the difference between vehicle-PBS and EAE animals, between Vehicle PBS and Dexamethasone treated animals, and between Vehicle for Blautix and Blautix-treated animals.

Serum from day -14 animals showed no IgG antibody responses to MOG 35-55 peptide, as expected for naïve animals. Serum from day 35 animals showed increased IgG antibody responses to MOG 35-55 in all EAE groups in comparison with control group animals (Figure 9). The increased antibody responses were not statistically significant, due to variability in antibody titres within each group, which is to be expected in the EAE model.

These data confirm that the disease induction achieved with the model is relevant to the pathology of autoimmune and inflammatory disorders of the central nervous system.

T cell proliferation

On Day 35, spleens from groups 2, 3, 8 and 9 were dissected out and processed to single cell suspensions. Splenocytes were cultured in the presence and absence of MOG 35-55 peptide for three days and tritiated thymidine incorporation was quantified to reveal levels of cell proliferation. Unstimulated and anti-CD3/anti-CD28 (positive control) stimulated control cultures were also established.

A two-way ANOVA followed by Sidak's post-test for multiple comparison was used to determine differences within *in vivo* treatment groups. A two-way ANOVA followed by Dunnett's post-test for multiple comparisons was used to determine differences within *ex vivo* treatment groups (media, MOG 35-55 or anti CD3/CD8). No significant differences were seen between Media and MOG 35-55 stimulated samples, however, between both media and MOG 35-55 and anti CD3/CD28 stimulation statistical significance was seen ($p < 0.0001$). This confirms ability of cells to produce a strong proliferative response following stimulation (data not shown).

MOG T cell proliferative responses in splenocytes at day 35 were increased above unstimulated (media alone) proliferative responses (Figure 10). Proliferative responses to an anti-CD3/CD28 stimulus were significantly increased ($p < 0.0001$), confirming the viability of the splenocytes and ability to proliferate strongly with a highly positive stimulus.

These data confirm that the disease induction achieved with the model is relevant to the pathology of autoimmune and inflammatory disorders of the central nervous system.

Example 2 – Effects of bacterial lyophilisate on SCFA production healthy rats

The effects of chronic administration of a lyophilisate of *Blautia hydrogenotrophica* strain DSM 14294 on SCFA production in healthy HIM rats were studied and the results are reported in Figure 6. Further details regarding the experiments are provided above in the descriptions of the figure. Figure 6 shows that administration of BH induces a significant increase in acetate as well as in butyrate production.

Example 3 – Efficacy of *B. hydrogenotrophica* studied in human Microbiota Associated rat (HMA rat) model

Summary

Groups of 16 germ-free rats (comprising 8 rats in the control group and 8 rats in the treatment group) were inoculated with the faecal microbiota from a human IBS subject (IBS-HMA rats). Three successive experiments were carried out using faecal samples from 3 different IBS patients. Two other groups of rats ($n = 10$) were inoculated with faecal samples of healthy subject (n=2 subjects; 2 groups of healthy-HMA rats) as visceral sensitivity control. Thus, there were 24 IBS-microbiota associated rats (control), 24 IBS microbiota associated rats treated with Blautix and 20 healthy-microbiota associated rats. Half of the IBS-HMA rats were then administered for 28 days with composition comprising the bacterial strain of *B. hydrogenotrophica* according to the invention while the other half animals received a control solution.

Strain

Blautia hydrogenotrophica (BH) strain DSM 14294.

Composition and administration

BH lyophilisate was suspended in sterile mineral solution to a concentration of 10^{10} bacteria per ml. Two ml of this suspension was administered daily per IBS-HMA rat, by oral gavage, for a 28 days period.

The control solution was the sterile mineral solution that was administered daily (2 ml per rat) by oral gavage to the control group of IBS-HMA rats.

Rats

Germ-Free male Fisher rats (aged 10 weeks) were inoculated with human faecal microbiota from an IBS subject (IBS-HMA rats). Sixteen rats were inoculated with the same human faecal inoculum. Three successive experiments were performed with faecal samples from three different IBS subjects. Two other groups of ten rats were inoculated with faecal sample from 2 healthy subjects (normo-sensitivity control groups).

Study design

Day -14 – Inoculation of Germ-free rats with human faecal microbiota.

Days 0 to 28 – Daily dose of BH lyophilisate (assay group), or control solution (control group) by oral gavage

Between days 14 and 22 – operation to implant electrode into the abdomen (for distension assay)

Days 22-28 – Adaptation of the rats to avoid stress associated with distension test.

Day 28 – distension assay and euthanasia of animals to collect the caecal samples for sulphides and short chain fatty acid (SCFA) analysis.

Days 0, 14 and 28 – Collection of faecal samples for microbial analysis: qPCR for evaluating BH population and other commensal groups of miccroorganisms and enumeration of functional groups of microorganisms using selective media and strictly anaerobic method.

Results

Figure 7 presents the results of qPCR analysis of the *B. hydrogenotrophica* population in faecal samples from IBS-HMA rats receiving control solution or BH lyophilisate. A significant increase in the BH population was observed at the end of the administration period (D 28) in rats receiving the BH lyophilisate, which confirms successful delivery of BH in the colon.

Figure 8 reports on the impact of administration of BH on the main fermentative metabolites, short chain fatty acids, in caecal samples of IBS-HMA rats. Administration of BH-resulted in a significant

increase in acetate concentration as well as in a significant increase in butyrate concentration (Figure 8B).

Example 4 – Stability testing

A composition described herein containing at least one bacterial strain described herein is stored in a sealed container at 25°C or 4°C and the container is placed in an atmosphere having 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90% or 95% relative humidity. After 1 month, 2 months, 3 months, 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, at least 50%, 60%, 70%, 80% or 90% of the bacterial strain shall remain as measured in colony forming units determined by standard protocols.

Sequences

SEQ ID NO:1 (*Blautia hydrogenotrophica* strain S5a36 16S ribosomal RNA gene, partial sequence - X95624.1)

```
   1 gatgaacgct ggcggcgtgc ttaacacatg caagtcgaac gaagcgatag agaacggaga
  61 tttcggttga agttttctat tgactgagtg gcggacgggt gagtaacgcg tgggtaacct
 121 gccctataca gggggataac agttagaaat gactgctaat accgcataag cgcacagctt
 181 cgcatgaagc ggtgtgaaaa actgaggtgg tataggatgg acccgcgttg gattagctag
 241 ttggtgaggt aacggcccac caaggcgacg atccatagcc ggcctgagag ggtgaacggc
 301 cacattggga ctgagacacg gcccaaactc ctacgggagg cagcagtggg gaatattgca
 361 caatggggga aaccctgatg cagcgacgcc gcgtgaagga agaagtatct cggtatgtaa
 421 acttctatca gcagggaaga aagtgacggt acctgactaa gaagccccgg ctaattacgt
 481 gccagcagcc gcggtaatac gtaaggggca agcgttatcc ggatttactg ggtgtaaagg
 541 gagcgtagac ggtttggcaa gtctgatgtg aaaggcatgg gctcaacctg tggactgcat
 601 tggaaactgt cagacttgag tgccggagag gcaagcggaa ttcctagtgt agcggtgaaa
 661 tgcgtagata ttaggaggaa caccagtggc gaaggcggcc tgctggacgg taactgacgt
 721 tgaggctcga aagcgtgggg agcaaacagg attagatacc ctggtagtcc acgctgtaaa
 781 cgatgaatac taggtgtcgg gtggcaaagc cattcggtgc cgcagcaaac gcaataagta
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1021 tggttgtcgt cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaaccct
1081 tattcttagt agccagcagg tagagctggg cactctaggg agactgccag ggataacctg
1141 gaggaaggtg gggatgacgt caaatcatca tgccccttat gatttgggct acacacgtgc
1201 tacaatggcg taaacaaagg gaagcgaagg ggtgacctgg agcaaatctc aaaaataacg
1261 tctcagttcg gattgtagtc tgcaactcga ctacatgaag ctggaatcgc tagtaatcgc
1321 gaatcagaat gtcgcggtga atacgttccc gggtcttgta cacaccgccc gtcacaccat
1381 gggagtcagt aacgcccgaa gtcagtgacc caaccnaaag gagggagctg ccgaaggtgg
1441 gactgataac tggggtga
```

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CLAIMS

1. A composition comprising a bacterial strain of the species *Blautia hydrogenotrophica*, for use in a method of treating or preventing an autoimmune or inflammatory disorder of the central nervous system.

2. The composition of claim 1, wherein the disorder is a demyelinating autoimmune disease.

3. The composition of claim 1, wherein the disorder is an inflammatory demyelinating disease.

4. The composition of claim 1, wherein the disorder is multiple sclerosis.

5. The composition of any preceding claim, wherein the composition is for use in a method of reducing disease incidence or disease severity.

6. The composition of any preceding claim, wherein composition is for use in preventing a decline in motor function or for use in improving motor function.

7. The composition of any preceding claim, wherein the bacterial strain has a 16s rRNA sequence that is at least 97%, 98%, 99%, 99.5% or 99.9% identical to SEQ ID NO:1 or which has the 16s rRNA sequence of SEQ ID NO:1.

8. The composition of any preceding claim, wherein the bacterial strain is the bacterium deposited under accession number DSM 14294.

9. The composition of any preceding claim, wherein the composition is for oral administration.

10. The composition of any preceding claim, wherein the composition comprises one or more pharmaceutically acceptable excipients or carriers.

11. The composition of any preceding claim, wherein the bacterial strain is lyophilised.

12. The composition of any preceding claim, wherein the bacterial strain is viable.

13. The composition of any preceding claim, wherein the composition comprises a single strain of the genus *Blautia.*

14. The composition of any preceding claim, which comprises the *Blautia hydrogenotrophica* bacterial strain as part of a microbial consortium.

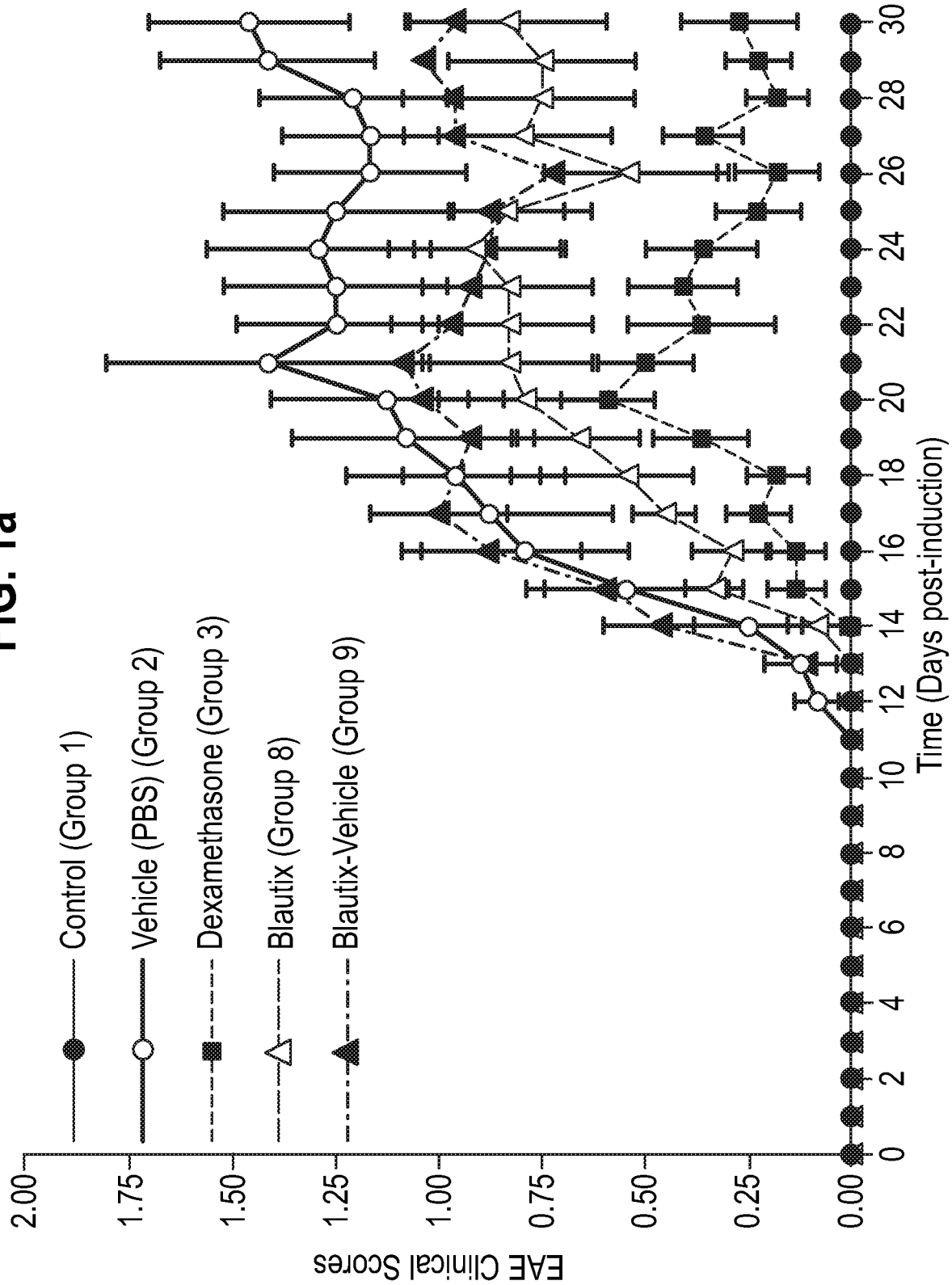
15. The composition of any preceding claim, which does not contain bacteria from any other species or which comprises only *de minimis* or biologically irrelevant amounts of bacteria from another species.

16. A food product comprising the composition of any preceding claim, for the use of any preceding claim.

17. A vaccine composition comprising the composition of any preceding claim, for the use of any preceding claim.

18. A method of treating or preventing an autoimmune or inflammatory disorder of the central nervous system, comprising administering a composition comprising a bacterial strain of the species *Blautia hydrogenotrophica* to a patient in need thereof.

FIG. 1a


Control (Group 1)
Vehicle (PBS) (Group 2)
Dexamethasone (Group 3)
Blautix (Group 8)
Blautix-Vehicle (Group 9)
EAE Clinical Scores
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Time (Days post-induction)

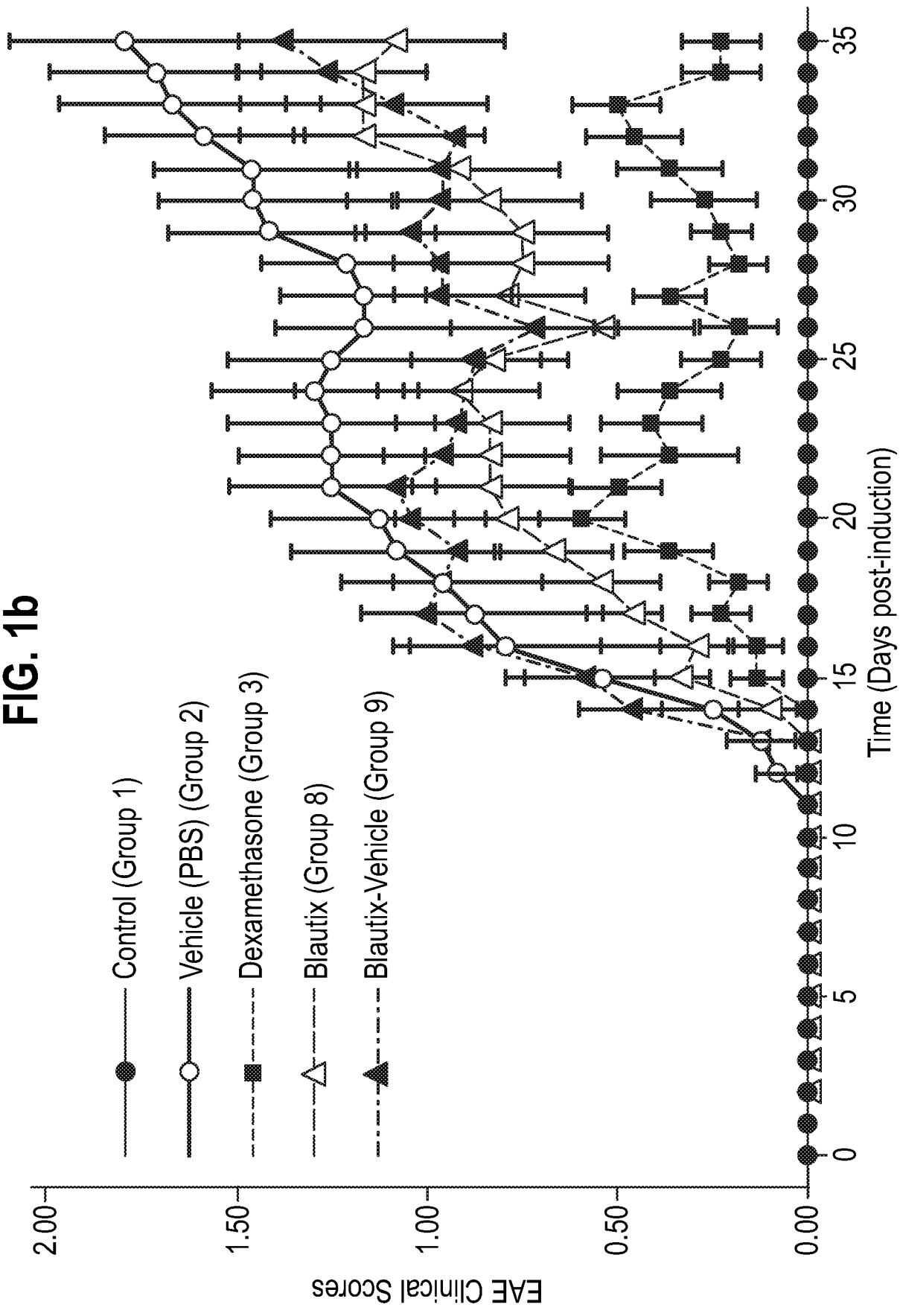

Control (Group 1)
Vehicle (PBS) (Group 2)
Dexamethasone (Group 3)
Blautix (Group 8)
Blautix-Vehicle (Group 9)
EAE Clinical Scores
2.00
1.50
1.00
0.50
0.00
0
5
10
15
20
25
30
35
Time (Days post-induction)

FIG. 1b

FIG. 2b

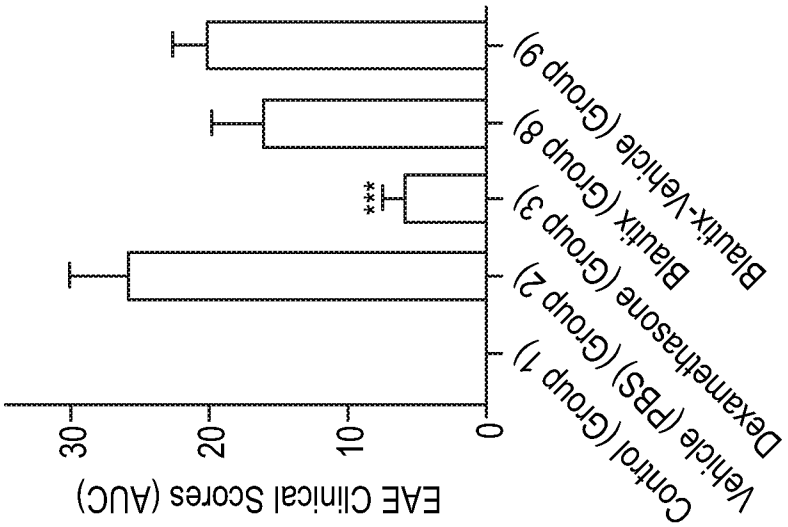

EAE Clinical Scores (AUC)
0
10
20
30
***
Control (Group 1)
Vehicle (PBS) (Group 2)
Dexamethasone (Group 3)
Blautix (Group 8)
Blautix-Vehicle (Group 9)

FIG. 2a

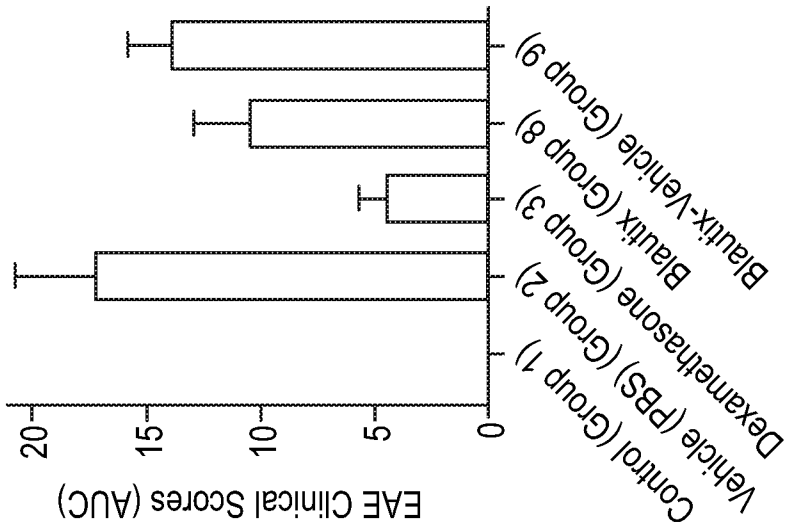

EAE Clinical Scores (AUC)
0
5
10
15
20
Control (Group 1)
Vehicle (PBS) (Group 2)
Dexamethasone (Group 3)
Blautix (Group 8)
Blautix-Vehicle (Group 9)

FIG. 3

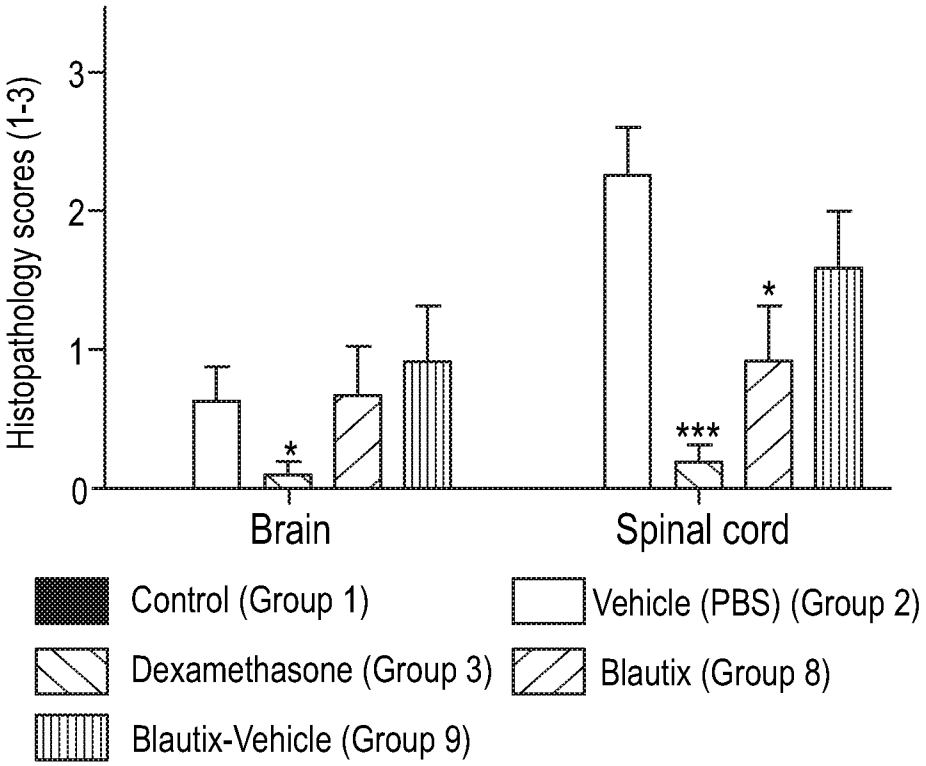

Histopathology scores (1-3)
3
2
1
0
*
***
*
Brain
Spinal cord
Control (Group 1)
Vehicle (PBS) (Group 2)
Dexamethasone (Group 3)
Blautix (Group 8)
Blautix-Vehicle (Group 9)

FIG. 6

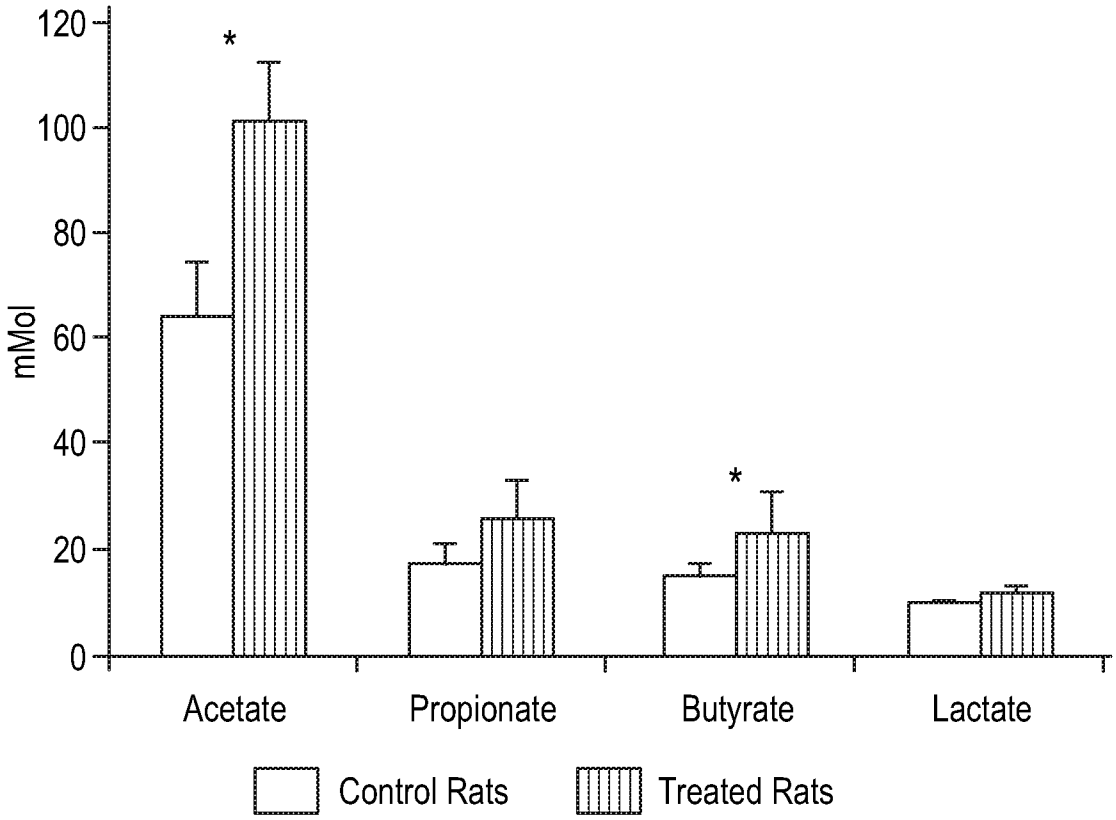
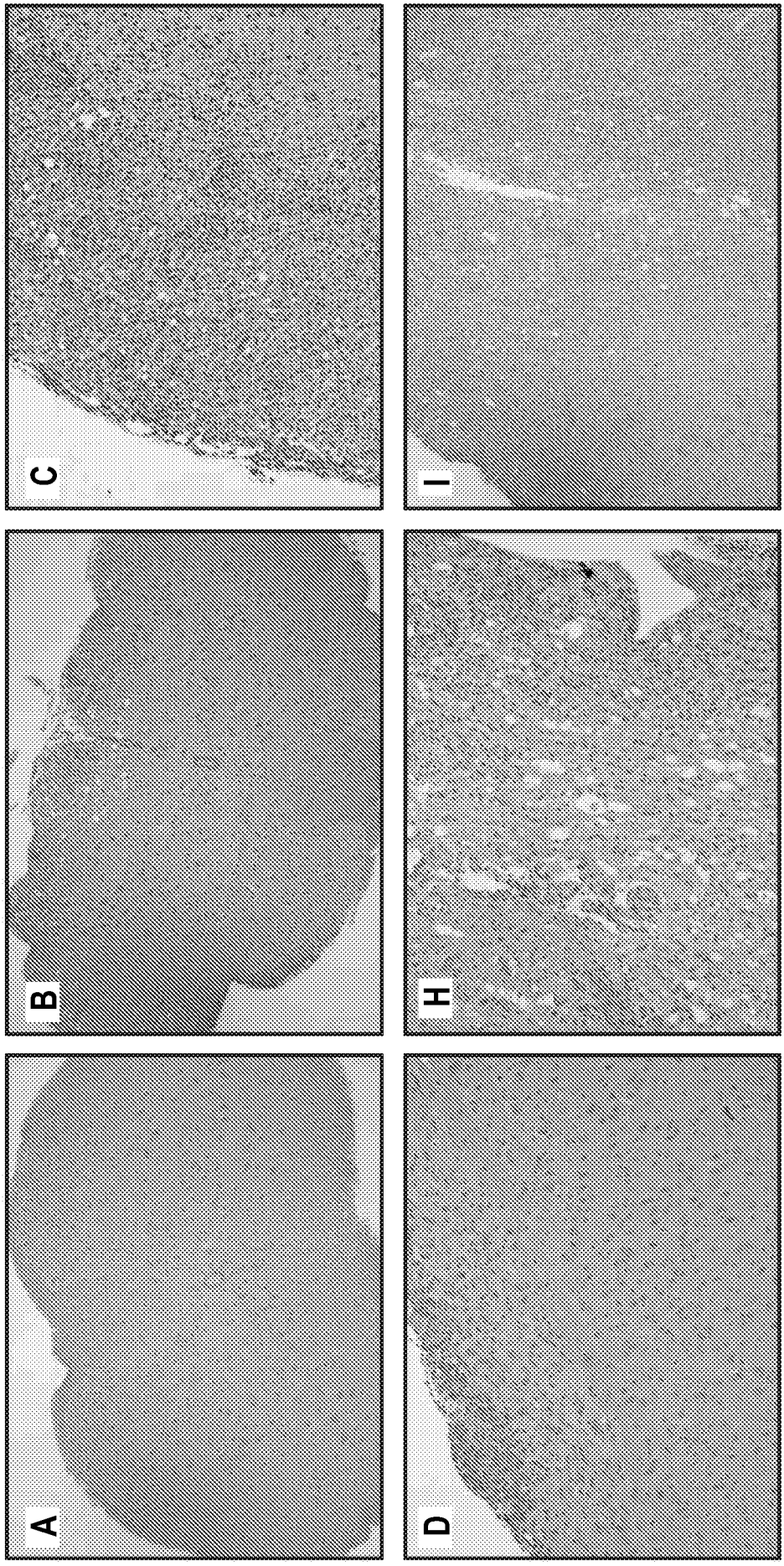

mMol
120
100
80
60
40
20
0
*
*
Acetate
Propionate
Butyrate
Lactate
Control Rats
Treated Rats

FIG. 4



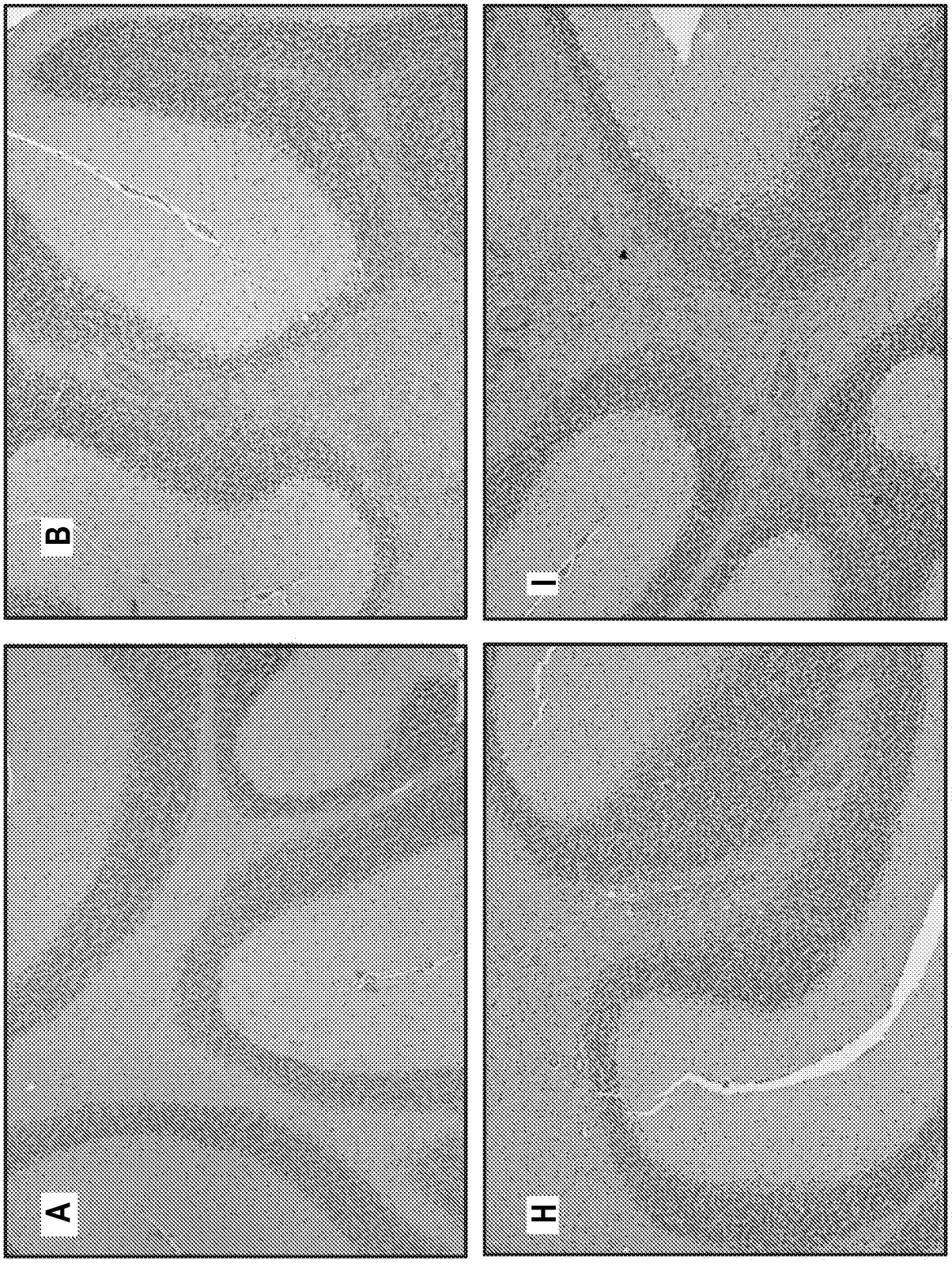


FIG. 5

FIG. 7

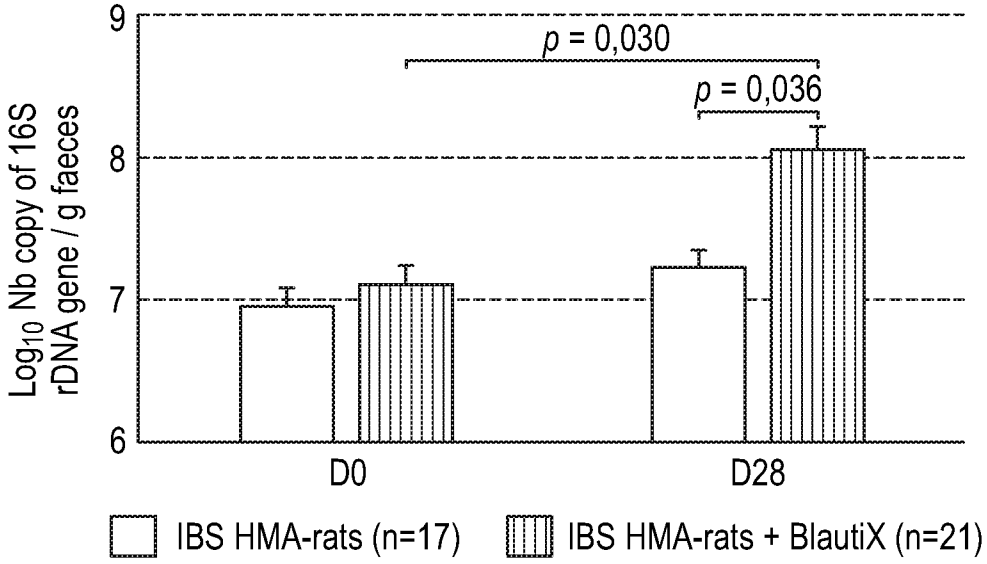

9
8
7
6
p = 0,030
p = 0,036
Log10 Nb copy of 16S rDNA gene / g faeces
D0
D28
IBS HMA-rats (n=17)
IBS HMA-rats + BlautiX (n=21)

FIG. 10

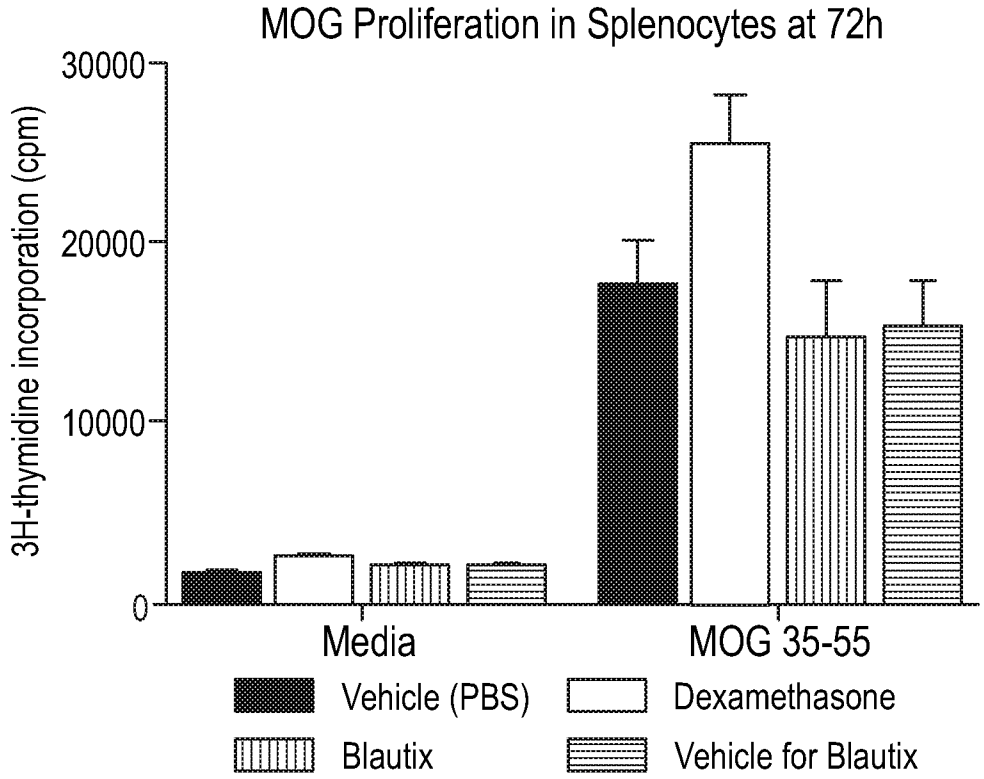

MOG Proliferation in Splenocytes at 72h
30000
20000
10000
0
3H-thymidine incorporation (cpm)
Media
MOG 35-55
Vehicle (PBS)
Dexamethasone
Blautix
Vehicle for Blautix

FIG. 8a

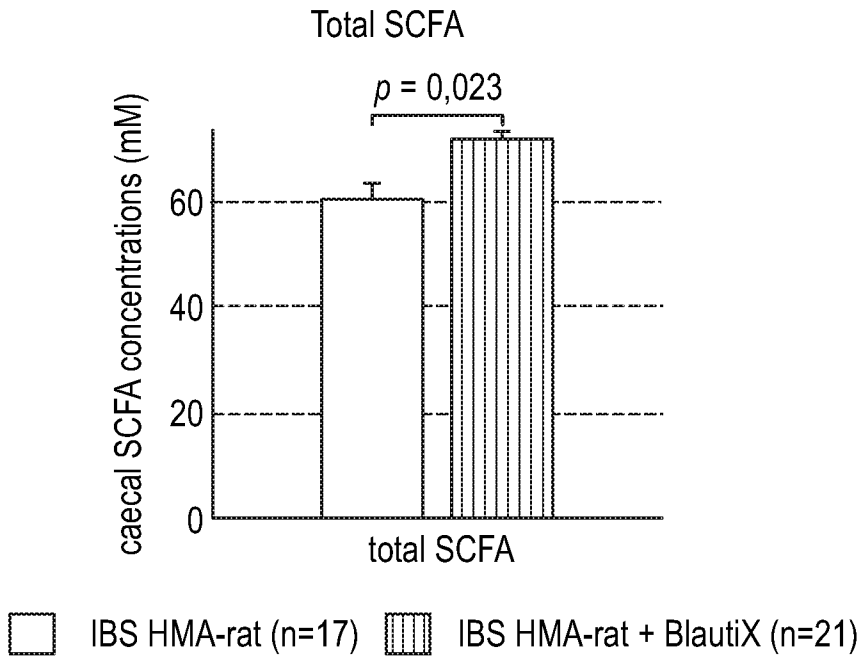

Total SCFA
p = 0,023
caecal SCFA concentrations (mM)
60
40
20
0
total SCFA
IBS HMA-rat (n=17)
IBS HMA-rat + BlautiX (n=21)

FIG. 8b

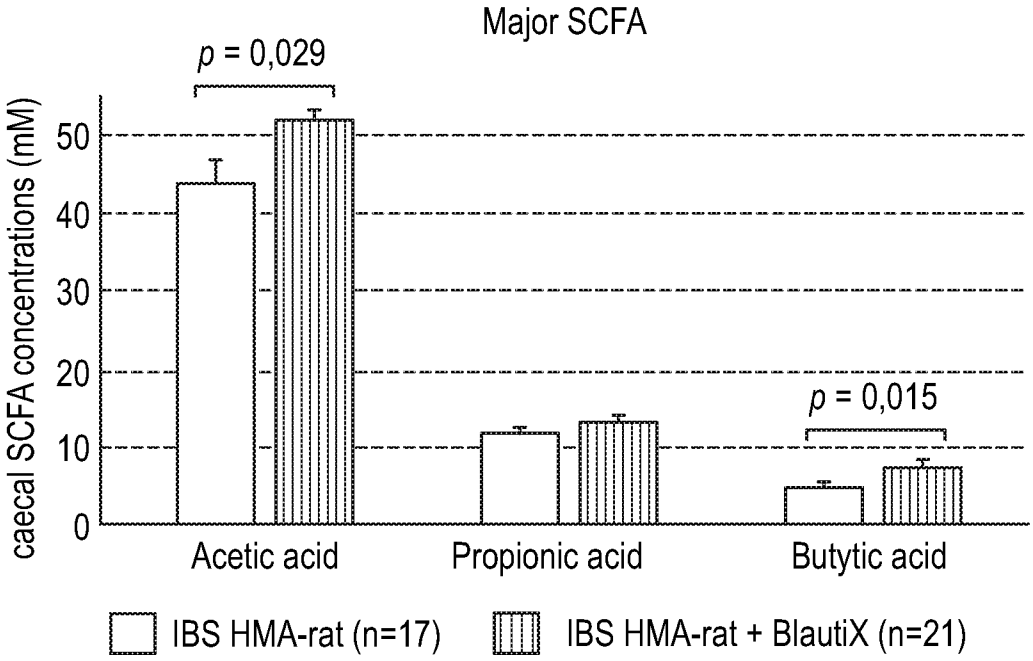

Major SCFA
p = 0,029
p = 0,015
caecal SCFA concentrations (mM)
50
40
30
20
10
0
Acetic acid
Propionic acid
Butytic acid
IBS HMA-rat (n=17)
IBS HMA-rat + BlautiX (n=21)

FIG. 9a

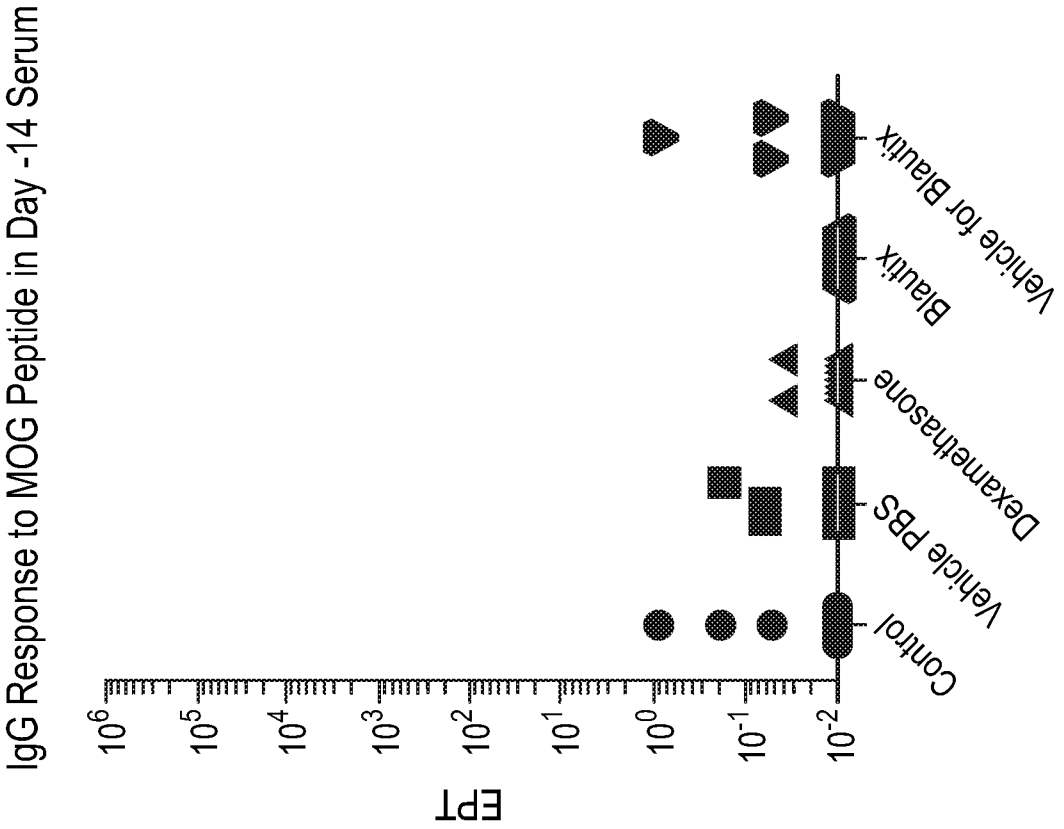
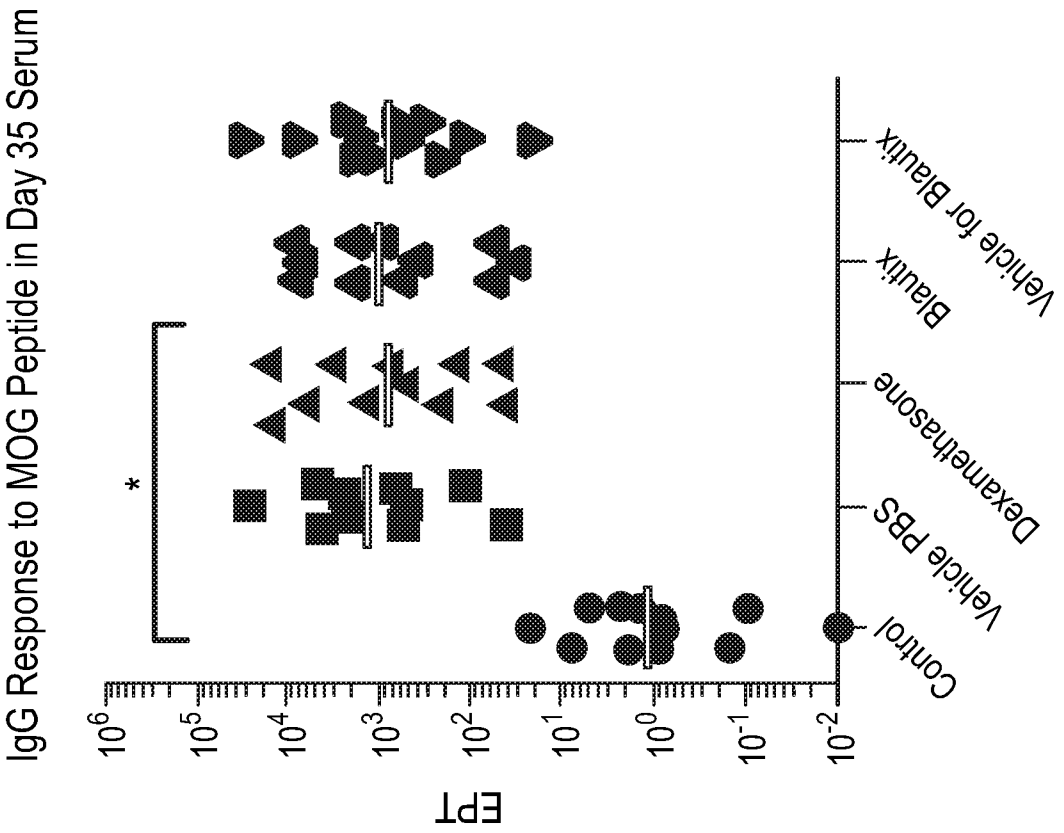

IgG Response to MOG Peptide in Day -14 Serum
EPT
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
Control
Vehicle PBS
Dexamethasone
Blaulix
Vehicle for Blaulix

FIG. 9b


IgG Response to MOG Peptide in Day 35 Serum
EPT
*
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
Control
Vehicle PBS
Dexamethasone
Blaulix
Vehicle for Blaulix

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/051391

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/74 A61P25/00 C12N1/20
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C12N

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2016/203218 A1 (4D PHARMA RES LTD [GB]) 22 December 2016 (2016-12-22) page 9, line 27 - page 14, line 4 page 19, paragraph 3 -----	1-6, 9-16,18 1-18
X Y	US 2016/143961 A1 (BERRY DAVID [US] ET AL) 26 May 2016 (2016-05-26) paragraphs [0017], [0165], [0270], [0277], [0355]; claims 3-7,21 -----	1-6, 9-16,18 1-18
Y	WO 2015/033305 A1 (SOFAR SPA [IT]) 12 March 2015 (2015-03-12) page 6, line 9 - line 28 -----	1-18
X,P	WO 2017/148596 A1 (4D PHARMA PLC [GB]) 8 September 2017 (2017-09-08) page 7, paragraph 3 -----	7-15
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 2018	08/08/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Zellner, Eveline

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2018/051391

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/051391

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BHARGAVA PAVAN ET AL: "Gut Microbiome and Multiple Sclerosis", CURRENT NEUROLOGY AND NEUROSCIENCE REPORTS, PHILADELPHIA, PA. : CURRENT SCIENCE INC, US, vol. 14, no. 10, 10 September 2014 (2014-09-10), pages 1-8, XP035394087, ISSN: 1528-4042, DOI: 10.1007/S11910-014-0492-2 [retrieved on 2014-09-10] abstract -----	1-18
A	C. LIU ET AL: "Reclassification of Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoides gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, vol. 58, no. 8, 1 August 2008 (2008-08-01) , pages 1896-1902, XP055005236, ISSN: 1466-5026, DOI: 10.1099/ijs.0.65208-0 abstract; figure 1 -----	1-18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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