The methods and compositions disclosed herein are useful in treatment and prevention of inflammatory bowel disease.


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**Abstract:** The present invention relates to methods for the treatment and/or prevention of bowel disorders. The methods and compositions disclosed herein are useful in treatment and prevention of inflammatory bowel disease.
METHODS AND COMPOSITIONS FOR THE TREATMENT AND/OR PREVENTION OF
BOWEL DISORDERS

FIELD OF THE INVENTION

[0001] The present invention relates to methods for the treatment and/or prevention of bowel disorders. The methods and compositions disclosed herein are useful in treatment and prevention of inflammatory bowel disease.

BACKGROUND OF THE INVENTION

[0002] Inflammatory bowel disease (IBD) is characterized by chronic or relapsing immune activation and inflammation within the gastrointestinal (GI) tract that markedly alters GI function. IBD includes Crohn's disease (CD) and Ulcerative Colitis (UC). CD manifests within the ileum and is characterized by multiple small areas of inflammation. CD has an early onset and commonly affects people aged 15-35. UC occurs in the colon and inflames large areas, ulcers are commonly observed. UC typically has a late onset and affects people aged 50-70 (Dvorak and Silen, 1985). Both of these conditions are chronic disorders with persistent spontaneous reoccurrence that significantly contribute to morbidity. Currently there is no successful treatment offered for either of these conditions other than surgery which removes large areas of the intestine affected by inflammation.

[0003] Inflammation of the gut is accompanied by a breakdown of intestinal barrier function, abnormal secretion, changes in the gut motility, and visceral sensation. These factors contribute to symptom generation. Standard IBD symptoms include diarrhoea, constipation, cramping, and abdominal pain,

[0004] Other chronic inflammatory diseases of the gut, including celiac disease, an autoimmune reaction to gluten found in wheat and other grains, and irritable bowel syndrome (IBS), are characterized by abdominal pain and GI dysfunction.

[0005] The enteric nervous system (ENS) is responsible for regulating vital digestive functions that contribute to both absorption of fluid and nutrients and maintenance of internal homeostasis. Additionally, the ENS is responsible for gut motility, the secretion of mucins, the production of cytokines, and the regulation of epithelial barrier function.

[0006] The ENS is more vulnerable than other areas of the nervous system to both physical and chemical damage due to its lack of protection and location within the digestive tract. Enteric neurons are susceptible to mechanical damage due to the function of the intestines
during digestion which must allow for the passage of digested matter. This is especially true for neurons within the colon as tissues in this area must stretch considerably to allow for fecal passage.

[0007] The ENS is also vulnerable to chemically induced damage as it is continually exposed to byproducts of digestion. Furthermore, the ENS contains neurons that extend their projections through the lamina propria. The lamina propria is an area that is abundant in cells of the immune system and their mediators, especially during bouts of inflammation.

[0008] Damage to enteric neurons incurred as a result of intestinal inflammation leads to abnormal electrophysiological properties of neurons throughout the gastrointestinal tract, which is thought to contribute to the symptoms of IBD outlined above. The exact mechanism that leads to enteric neuronal damage is unknown however alterations in electrophysiological properties of these neurons is commonly seen and can lead to changes in secretion, motility and gastrointestinal sensation.

[0009] Alterations that occur throughout the gastrointestinal tract during inflammation are not limited to electrophysiological properties of neurons, changes in epithelial composition are also commonly observed. Changes in immune cell population and distribution in the enteric ganglia have also been reported. These changes persist long after the resolution of inflammation.

[0010] The reason behind the inflammation seen in UC and CD is unknown. However during bouts of inflammation the typical inflammatory immune response is evoked. The walls of blood vessels become more permeable and allow inflammatory cells and mediators to leak out into inflammatory sites. Chemoattractants are released during this phase to attract immune cells to the affected areas. Cells such as neutrophils, lymphocytes, mast cells, eosinophils accumulate at the site of inflammation. Some of these cells degranulate in response to inflammation to upregulate the inflammatory reaction. All of these cells have been identified in histological analysis of tissues during intestinal inflammation. These cells are generally found to be within the lamina propria and myenteric plexus. Consequently, the available data suggest that that immune cells and/or their mediators are responsible for damage caused to the ENS during inflammation.

[0011] Treatments available for IBD at present are invasive and limited. Often surgical resections are performed on IBD patients to remove inflamed sections of the gastrointestinal tract, however this can only be done maximally 3-5 times. After this patients are treated with
corticosteroids alone which does little to alleviate the symptoms of IBD and instead suppresses the immune system which predisposes patients to infections and malignancies.

[0012] The modulation of inflammation of the ENS, or neuroinflammation, represents an unmet need in the treatment of inflammatory bowel disease. Surprisingly, the inventors have found that bowel disorders may be treated and/or prevented by administration of compounds which modulate enteric function controlled by the ENS. These compounds can also modulate inflammation associated with the ENS.

SUMMARY OF THE INVENTION

[0013] In an aspect of the invention there is provided a method of treating and/or preventing a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function.

[0014] According to a further embodiment the bowel disorder is inflammatory bowel disease or post-inflammatory irritable bowel syndrome.

[0015] According to a further embodiment the modulator of ENS-controlled enteric function modulates inflammation associated with the ENS.

[0016] According to a further embodiment the modulator of ENS-controlled enteric function modulates the accumulation of eosinophils in the gut. In a preferred embodiment, the modulator modulates the accumulation of eosinophils in the myenteric plexus. In a preferred embodiment, the eosinophils are eosinophil derived neurotoxin (EDN)-positive. In a preferred embodiment, the modulator of ENS-controlled enteric function modulates the accumulation and/or activation of eosinophils.

[0017] According to a further embodiment, the modulator of ENS-controlled enteric function is a modulator of CCR3 activity and/or an Eotaxin-1 activity.

[0018] In a further embodiment, the modulator is administered at a dose sufficient to antagonise CCR3 activity. In a preferred embodiment, the modulator is administered at a dose of at least 1mg/kg.
[0019] In a further embodiment the modulator is administered at a dose sufficient to antagonise CCR3 activity and eotaxin-1 activity. In a preferred embodiment, the modulator is administered at a dose of at least 3 mg/kg. According to a further embodiment, the modulator of ENS-controlled enteric function is SB-328437.

[0020] In a further embodiment, the modulator of ENS-controlled enteric function is an antioxidant. In a preferred embodiment, the modulator is resveratrol. In a preferred embodiment, resveratrol is administered at a dose of at least 0.1 mg/kg/d.

[0021] According to another aspect, the present invention provides a use of a modulator of ENS-controlled enteric function in the preparation of a medicament for the prevention and/or treatment of a bowel disorder. In a preferred embodiment the bowel disorder is inflammatory bowel disease or post-inflammatory irritable bowel syndrome. In a preferred embodiment, the modulator of an ENS-controlled enteric function is a modulator of CCR3 activity and/or an Eotaxin-1 activity. In a preferred embodiment, the modulator is formulated for administration at a dose of at least 1 mg/kg. In a preferred embodiment, the modulator is a CCR3 antagonist and/or an eotaxin-1 antagonist. In a preferred embodiment, the modulator is formulated for administration at a dose of at least 3 mg/kg. According to a preferred embodiment, the modulator of ENS-controlled enteric function is SB-328437. According to a further embodiment, the modulator of ENS-controlled enteric function is resveratrol. In a further embodiment, the modulator is resveratrol formulated for administration at a dose of at least 0.1 mg/kg/d.

[0022] According to another aspect, the present invention provides a composition comprising a modulator of an ENS-controlled enteric function when used for the treatment and/or prevention of a bowel disorder. In a preferred embodiment the bowel disorder is inflammatory bowel disease or post-inflammatory irritable bowel syndrome. In a preferred embodiment, the modulator of an ENS-controlled enteric function is a modulator of CCR3 activity and/or an Eotaxin-1 activity. In a preferred embodiment, the modulator is administered at a dose of at least 1 mg/kg. In a preferred embodiment, the modulator a CCR3 antagonist and/or an eotaxin-1 antagonist. In a preferred embodiment, the modulator is administered at a dose of at least 3 mg/kg. According to a preferred embodiment, the modulator of ENS-controlled enteric function is SB-328437. According to a further embodiment, the modulator of ENS-controlled enteric function is resveratrol. In a further embodiment, the modulator is resveratrol is administered at a dose of at least 0.1 mg/kg/d.
The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 shows analysis of speed propagation of spatiotemporal maps of gut width. Speed of propagation is measured by taking the gradient of the contraction (as pictured), and the value is given in mm/sec. The speed of each whole length contraction per 10 minute recording was measured and averaged.

Figure 2 shows analysis of quiescence (time interval between contractions) and amplitude of contractions of spatiotemporal maps of gut width. Analysis of quiescence (time interval between contractions) and amplitude of contractions required 3 cross sections to be taken 10mm apart and evaluated. Cross sections were converted into graphical form for evaluation using Matlab.

Figure 3 shows determination of quiescence (time interval between contractions) of gut contractions. Quiescence (in seconds) was determined by taking the time values at the bottom of the contraction peaks and subtracting them from one another to determine the difference. The final values were averaged and used for concluding results.

Figure 4 shows determination of amplitude of gut contractions. The values for amplitude (mm) were obtained by taking the values from the top and bottom of the contraction peaks and subtracting them from one another appropriately to determine the difference (amplitude).
[0029] Figure 5 shows activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric plexus and neuronal processes of the enteric nervous system in inflammatory bowel disease, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus and neuronal processes of the enteric nervous system.

[0030] Confocal images x60 magnification showing EDN-positive cell populations (red) within the myenteric plexus and neuronal processes (green). (A) shows low levels of EDN-positive cells in control tissue (B) shows significant accumulation of EDN-positive cells following TNBS treatment (C) shows abundance of EDN-positive cells close to myenteric ganglia following TNBS + vehicle treatment (D) shows low levels of EDN-positive cells similar to control tissue following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus and neuronal processes of the enteric nervous system. Scale equals 100 µm. Arrows point to myenteric ganglia pictured in green and EDN cells pictured in red.

[0031] Figure 6 shows inflammation induced damage to myenteric ganglia and neuronal processes in inflammatory bowel disease, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents damage to myenteric ganglia and neuronal processes.

[0032] Confocal images x20 showing damage to myenteric ganglia and neuronal processes (green) in guinea pig colon. (A) shows normal appearance of ganglia and neuronal processes (B) shows damage to ganglia and loss of neuronal processes following induction of inflammatory bowel disease using TNBS (C) shows deformed ganglia and loss of neuronal processes following TNBS + vehicle treatment (D) shows intact ganglia and normal appearance of processes similar to control tissue following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating administration of CCR3 antagonist prevents inflammation induced damage to myenteric ganglia and neuronal processes. Scale equals 100 µm. White arrows point to myenteric ganglia and pink arrows point to neuronal processes pictured in green.
[0033] Figure 7 shows inflammation induced damage to neuronal projections within the mucosa, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents inflammation induced damage to neuronal projections within the mucosa.

[0034] Fluorescent images x20 magnification showing inflammation induced damage to neuronal projections within the mucosa stained with β-tubulin. (A) shows normal appearance of neuronal processes within mucosa in control tissue (B) shows partial loss of neuronal processes of following TNBS treatment, 24 hours after TNBS administration (C) shows almost complete loss of neuronal projections following TNBS + vehicle treatment (D) shows reduced loss of neuronal projections following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating treatment with CCR3 antagonist before induction of inflammatory bowel disease reduces inflammation induced damage to neuronal projections within the mucosa. Arrows point to neuronal projections within mucosa.

[0035] Figure 8 shows inflammation induced changes to histological structure of guinea pig colon, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease reduces transmural inflammation and mucosal insult.

[0036] Images taken at x20 magnification showing standard H&E staining of guinea pig colon. (A) Sham animal tissue showing normal appearance of colon tissue (B) TNBS animal tissue showing transmural inflammation and mucosal insult (C) TNBS + vehicle tissue showing transmural inflammation and mucosal insult (D) TNBS + CCR3 tissue showing reduced transmural inflammation and mucosal insult following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating treatment with CCR3 antagonist before induction of inflammatory bowel disease reduces transmural inflammation and mucosal insult.

[0037] Figure 9 shows quantification of EDN-positive cell accumulation within the myenteric plexus at 1 and 7 days following induction of inflammatory bowel disease using TNBS treatment, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus of the enteric nervous system.
[0038] Quantification of EDN positive cells within the myenteric plexus per cm² 1 and 7 days post TNBS treatment ("1 D" and "7D", respectively) in animals following treatment with TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). *** P<0.001 significantly different from all other groups. This data shows that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus of the enteric nervous system.

[0039] Figure 10 shows enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility.

[0040] The average number of contractions per minute are shown 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following treatment with TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). * P<0.05 significantly different from all other groups. ** P<0.001 significantly different from all other groups. *** P<0.0001 significantly different from all other groups. This data shows that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility.

[0041] Figure 11 shows enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility at different levels of intraluminal pressure.

[0042] The average number of contractions per minute are shown 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following treatment with TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). The data is shown for basal pressure ("base"), 1 cm above basal pressure ("+1"), 2 cm above basal pressure ("+2"), and 3 cm above basal pressure ("+3"). This data shows that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility.
[0043] Figure 12 shows enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility at different levels of intraluminal pressure.

[0044] The length of contractions per minute are shown 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following treatment with TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). The data is shown for 1 cm above basal pressure ("1"), 2 cm above basal pressure ("2"), 3 cm above basal pressure ("3"), and 4 cm above basal pressure ("4").

[0045] Figure 13 shows enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease, that treatment with CCR3 antagonist before or after induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility, and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0046] The total number of the colon whole length contractions induced by 3mm of intraluminal pressure per minute are shown 1 and 7 days post TNBS treatment ("Day1" and "Day7", respectively) in sham treated animals ("Shams"), following treatment with TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("PreTNBS"), treatment with CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment ("Post TNBS"), and treatment with Resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Resveratrol"). This data shows that treatment with CCR3 antagonist prior to or following, or treatment with resveratrol prior to, induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. This data also shows administration of a high dose of CCR3 antagonist prevents reduction of enteric nervous system controlled intestinal motility.

[0047] Figure 14 shows the survival of enteric neurons in the myenteric plexus is reduced following induction of inflammatory bowel disease, and that treatment with CCR3 antagonist
or Resveratrol after induction of inflammatory bowel disease promotes survival of enteric neurons in the myenteric plexus.

[0048] The number of enteric neurons are shown 1 and 7 days post TNBS treatment ("1 D" and "7 D", respectively) in sham treated animals ("Sham"), following treatment with TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS, and treatment with Resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Resveratrol"). This data shows that treatment with CCR3 antagonist or resveratrol prior to induction of inflammatory bowel disease promotes survival of enteric neurons in the myenteric plexus. This data also shows administration of a high dose of CCR3 antagonist promotes survival of enteric neurons in the myenteric plexus.

[0049] Figure 15 shows activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric ganglia of the enteric nervous system in inflammatory bowel disease, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric ganglia.

[0050] Wholemount preparations x20 and x 40 magnification showing EDN-positive cell populations (red) within the myenteric ganglia labelled with anti-B-Tubulin antibody (green) attached to the longitudinal muscles. EDN-positive cells labelled with anti-EDN antibody (red) found at the level of myenteric plexus. Significant accumulation of EDN-positive cells following TNBS treatment are shown at X20 and x40 magnification, and low levels of EDN-positive cells similar to following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment. This demonstrates treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric ganglia of the enteric nervous system.

[0051] Figure 16 shows activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present the colon in inflammatory bowel disease, and that treatment with high or low doses of CCR3 antagonist before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0052] Immunohistochemical analysis of the number of EDN-positive cells in cross sections of the guinea-pig colon from the animals treated with CCR3 antagonist and Resveratrol. EDN-positive cells were labelled using anti-EDN antibody (red) and neuronal processes were
labelled with anti-B-Tubulin antibody (green). This data shows that activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present the colon in inflammatory bowel disease, and that treatment with high or low doses of CCR3 antagonist prior to or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon. This data also demonstrates resveratrol had no significant effect on the number of eosinophil-derived neurotoxin (EDN)-positive cells in the cross sections of the colon.

[0053] Figure 17 shows quantification of EDN-positive cell accumulation within the colon at day 1 following induction of inflammatory bowel disease using TNBS treatment, and that treatment with CCR3 antagonist before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0054] Quantification of EDN positive cells within the colon per cm² at 1 day post TNBS treatment (“1 D”) in animals following treatment with TNBS only (“TNBS”), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment (“CCR3 + TNBS”), and following administration of CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment (“TNBS + CCR3”). P<0.05, significantly different from all other groups. This data shows that treatment with CCR3 antagonist before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0055] Figure 18 shows quantification of EDN-positive cell accumulation within the colon at day 7 following induction of inflammatory bowel disease using TNBS treatment, that treatment with high or low dose CCR3 antagonist prior to induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0056] Quantification of EDN positive cells within the colon per cm² at day 7 post TNBS treatment (“7D”) in animals following treatment with TNBS only (“TNBS”), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment (“CCR3 + TNBS”). P<0.05, significantly different from TNBS group. This data shows that treatment with CCR3 antagonist prior to induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon, and that resveratrol had no significant effect on the number of eosinophil-derived neurotoxin (EDN)-positive cells in the cross sections of the colon. This data also shows administration of a high dose of CCR3 antagonist prevents accumulation of activated eosinophils within the colon.

[0057] Figure 19 shows enteric nervous system controlled intestinal motility is reduced
following induction of inflammatory bowel disease, that treatment with CCR3 antagonist before or after induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility, and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0058] The rate of contractions per minute are shown 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in sham treated animals ("Sham"), following treatment with TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("Pre"), treatment with CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment ("Post"), and treatment with resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Res"). This data shows that treatment with CCR3 antagonist prior to or following, or treatment with resveratrol prior to, induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. This data also shows administration of a high dose of CCR3 antagonist prevents reduction of enteric nervous system controlled intestinal motility.
DETAILED DESCRIPTION OF THE INVENTION

[0059] The present invention is based in part on the demonstration that modulation of enteric nervous system controlled enteric function prevents and/or treats a disorder of the bowel.

[0060] Accordingly, in a first aspect of the present invention, there is provided a method of treating and/or preventing a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function.

[0061] As used herein, the enteric nervous system refers to a collection of neurons in the gastrointestinal tract. These neurons are grouped together into small ganglia connected by bundles of nerve processes forming the two major ganglionated plexuses, the myenteric plexus and submucosal plexus, respectively. The two plexuses differ by both location and function. The myenteric plexus is situated within the intestinal walls between the circular and longitudinal muscle layers and is responsible for propulsion of digested matter along the intestine (motility). The submucosal plexus exists beneath the circular muscle layer and above the muscularis mucosae and its function is to regulate secretion and absorption. Accordingly, an ENS-controlled enteric function includes gut motility (e.g. Speed of propagation, time interval of contractions (quiescence), amplitude of contractions, and length of contractions), fluid exchange, local blood flow, gastric and pancreatic secretion, including secretion of enzymes, mucin, hormones and other compounds.

[0062] Enteric neurons are regularly separated into two subclasses based on function, morphology and electrophysiological properties. Neurons are either classed as synaptic (S) or afterhyperpolarising (AH) neurons. S neurons have Dogiel type I morphology which means they are monoaxonal. Functionally, S neurons are interneurons or motor neurons. These neurons receive fast synaptic inputs. They are referred to as compound neurons as synaptic transmission is of the result of summed inputs from several connecting neurons. These neurons exhibit brief action potential (AP) followed by short duration afterhyperpolarising potential (AHP) which lasts 20-100ms. AH neurons have Dogiel type II morphology which means they are multiaxonal. Functionally AH neurons are primary intrinsic afferent neurons. AH neurons are typically less excitable than S neurons. Their APs are of greater duration and higher amplitude with a characteristic hump on the falling phase. They show two distinct phases of afterhyperpolarisation which contribute to their low level of excitability. Early AHP lasts 20-100ms and late AHP has a duration of 4-25s. Late AHP is caused by opening of intermediate conductance calcium sensitive potassium channels. These neurons usually
show slow excitatory postsynaptic potentials (EPSP) especially within guinea pig ileum, which is enough to produce an AP.

[0063] Gastrointestinal inflammation results in altered functions of enteric neurons which affects normal intestinal motility, secretion and sensation. The mechanism by which inflammation induces changes to enteric neurons is still unknown. Damage to enteric neurons results in changes in neuronal excitability, synaptic transmission and electrophysiological properties of neurons. The changes in electrophysiological properties lead to irregularities of gastrointestinal functions and may contribute to the symptoms observed in IBD.

[0064] Inflammation of the gut results in altered electrophysiological properties of enteric neurons. Neurons affected by inflammation become more excitable (hyperexcitable). Hyperexcitable neurons show a more depolarised resting membrane potential (RMP) which means their RMP lies closer to threshold for induction of AP than normal. As a consequence of this they also exhibit a lower threshold for evoking a response to a stimulus and more action potentials observed at the same frequency as compared to sham operated controls. The main subclasses of neurons affected by such changes are Dogiel type II 'AH' neurons. Hyperexcitability of AH neurons is characterised by a reduced amplitude of AHP (in normal conditions AHP contributes to low excitability of these neurons). AH neurons were additionally reported to receive fast EPSP in animals with TNBS induced inflammation, which are not typical for them in normal condition.

[0065] In addition to altered functional properties of enteric neurons in response to inflammation, various studies have demonstrated that enteric inflammation generates structural abnormalities to the ENS including morphological changes and neuronal cell death.

[0066] Accordingly, there exists a need for neuroprotective agents that can mitigate the effects of an inflammatory insult on the ENS and thereby prevent and/or treat gut dysfunction associated with bowel disorders including IBD and post-inflammatory IBS.

[0067] The inventors have demonstrated that use of certain compounds described herein can protect enteric neurons from inflammation-associated damage. These compounds have also been demonstrated to modulate enteric function controlled by the ENS. The inventors have found that administration of compounds which modulate enteric function which is controlled by the ENS may be used in the treatment and/or prevention of bowel disorders.
Accordingly in an embodiment of the invention, the bowel disorder which may be prevented and/or treated is inflammatory bowel disease or post-inflammatory irritable bowel syndrome.

The term "disease" or "disorder," as used herein, refers to an impairment of health or a condition of abnormal functioning.

The term "inflammatory bowel disease" as used herein has its usual medical meaning, and refers to diseases including, but not limited to, Crohn's disease, ulcerative colitis, Johne's disease, Behget's syndrome, collagenous colitis, diversion colitis, indeterminate colitis, infective colitis, ischaemic colitis, lymphocytic colitis, and closely related diseases and disorders of the gastrointestinal tract.

As used herein, a "therapeutically effective amount" of a modulator of an enteric nervous system (ENS)-controlled enteric function refers to an amount of the modulator which is effective, upon single or multiple dose administration to a subject (such as a human patient) at treating, preventing, curing, delaying, reducing the severity of, and/or modulating at least one symptom of a condition, disease or disorder, or prolonging the survival of the subject beyond that expected in the absence of such treatment. As a non-limiting example, the term "effective amount" refers to a dosage or amount that is sufficient to modulate clinical symptoms, e.g. restoration of normal gut motility or frequency of diarrhoea, or to achieve a desired biological outcome, e.g. such as through reduced inflammation, reduced accumulation of EDN, MBP etc.

A modulator of an enteric nervous system (ENS)-controlled enteric function may be determined by methods known to the skilled addressee, such as those set forth in Examples 8 to 11 and 19.

As used herein, the terms "treat," "treating" and "treatment" refer to the eradication, reduction or amelioration of symptoms of a condition, disease or disorder. As used herein, the term "amelioration" means that signs or symptoms associated with the condition, disease or disorder are lessened. In certain embodiments, such terms refer to the minimizing or delaying the spread of disease or the eradication, removal, modification or control of disease resulting from the administration of one or more therapeutic agents to a subject with such a disease.
[0074] As used herein, the terms "prevent," "preventing" and "prevention" refer to the prevention of the occurrence, recurrence or spread of a condition, disease or disorder in a subject resulting from the administration of a prophylactic or therapeutic agent.

[0075] As used herein, the term "modulate" or "modulating" refers to adjusting, changing, or manipulating, for example an increase or decrease, the amount, quality, response or effect of a particular activity, function or molecule. Accordingly, a "modulator" is an agent that modulates.

[0076] As used herein, the term "antagonist" refers to any molecule that is capable of blocking or decreasing the amount of ligand binding to a receptor. An antagonist may decrease receptor/ligand binding by interacting with either the receptor or ligand. An antagonist is capable of diminishing or abolishing receptor/ligand interactions.

[0077] The term "individual" is meant to include an individual organism, preferably a vertebrate, more preferably a mammal, preferably a human. In some embodiments, the individual is human, including adults, children and premature infants. In some variations, the primate is a non-human primate such as a chimpanzee, another species of ape, or a monkey. In some embodiments, the mammal is a farm animal such as a cow, a horse, a sheep, a goat, or a pig. In other variations the mammal is a companion animal such as a rabbit, a dog, or a cat; a laboratory animal such as a member of the rodent family, which includes rats, mice, and guinea pigs; and the like. In some embodiments, the individual is a non-mammal. Examples of non-mammals include, but are not limited to, birds, reptiles, amphibians, and the like. The term "individual" does not denote a particular age or sex.

[0078] In some variations, the individual has been identified as having one or more of the conditions described herein. Identification of the conditions as described herein by a skilled physician is routine in the art and may also be suspected by the individual or others.

[0079] In some embodiments, the individual has been identified as susceptible to one or more of the conditions as described herein. The susceptibility of an individual may be based on any one or more of a number of risk factors and/or diagnostic approaches appreciated by the skilled artisan, including, but not limited to, genetic profiling, family history, medical history (e.g., appearance of related conditions), lifestyle, or habits.

[0080] As shown in examples 4, 5 and 14, the inventors have demonstrated that inflammation induced damage to the myenteric ganglia, neuronal processes and neuronal projections
within the mucosa is elevated in inflammatory bowel disease and reduced following
administration of a modulator of the present invention either prior to or after induction of
inflammatory bowel disease. Specifically, the inventors have demonstrated that
administration of certain modulators prior to induction of inflammatory bowel disease
promotes survival of enteric neurons in the myenteric plexus.

[0081] Accordingly, in an embodiment of the invention, the modulator of ENS-controlled
enteric function modulates inflammation induced damage to the ENS.

[0082] Mast cells, lymphocytes, neutrophils and eosinophils all accumulate in response to
inflammation. Mast cells secrete histamine, prostaglandins and leukotrienes all of which play
a role in the up-regulation of inflammation. Exogenous histamine, leukotrienes and
prostaglandins applied directly to neurons enhance excitability. Histamine and prostaglandins
can also act presynaptically to diminish the sympathetic nervous system's inhibitory input to
secretomotor neurons. This action is more pronounced during inflammatory states and can
lead to hypersecretion which augments diarrhoea.

[0083] The precise mechanism of induction of neuronal cell death remains elusive; however,
the loss of neurons is associated with the appearance of eosinophilic and neutrophilic
infiltrates as well as infiltrates of lymphocytes such as plasma cells and mast cells into
myenteric plexus.

[0084] These changes induced by inflammation of the gut lead to significant alterations to the
functions of the gut controlled by the ENS. Importantly, these changes have been
demonstrated to persist following resolution of the inflammatory stimulus, suggesting that
earlier inflammation can alter gut function (such as in post-inflammatory irritable bowel
syndrome).

[0085] As previously mentioned, a number of different cell types accumulate in the gut in
response to an inflammatory stimulus. In this context, one cell type which has been the focus
of investigation for its role in injury to the ENS is the neutrophil. Indeed, a timeline that
correlated neutrophil accumulation to the specific time point when most damage or loss of
enteric neurons occurred was established implicating neutrophils in inflammation-induced
alterations to enteric neurons. Additionally, blockade of neutrophil accumulation was shown to
partially attenuate neuronal loss over a similar time course. This finding suggests that
neutrophils are implicated but are not solely responsible for damage incurred to enteric
neurons. Other immune cells or mediators might act in conjunction with neutrophils to
produce this effect. Lymphocytes are undeniably involved in the pathogenesis but proven to be an inappropriate target for therapeutic intervention.

[0086] Eosinophils are pro-inflammatory leukocytes that constitute a small percentage of circulating blood cells and are one of a panoply of cells that migrate to sites of inflammation in the gut. Eosinophils secrete toxic inflammatory mediators that are both stored in intracellular granules and also synthesised upon cellular activation. Key proteins secreted by eosinophils include eosinophilic cationic protein, major basic protein (MBP), eosinophil protein X, eosinophil derived neuroendotoxin (EDN), and eosinophil peroxidase. These cause damage to tissues, insert pores into membranes of target cells, and increase smooth muscle reactivity by generating toxic oxygen radicals.

[0087] Eosinophils migrate to the gastrointestinal tract in response to chemoattractant molecules, or chemokines. One key chemokine involved in this recruitment is eotaxin, a chemokine that is constitutively expressed throughout the gastrointestinal tract. Eotaxin binds to the CCR-3 receptor on eosinophils and is required for their homing to the gastrointestinal tract.

[0088] However, constitutive expression of eotaxin is not sufficient for tissue eosinophil trafficking because some segments of the gastrointestinal tract (e.g. tongue and oesophagus) express eotaxin yet are normally devoid of eosinophils. Therefore, it is apparent that other mediators are required to complete the homing of eosinophils to the gut. For example, one such mediator is the pro-inflammatory cytokine IL-5. This cytokine has been shown to increase the circulating pool of eosinophils and primes eosinophils to have enhanced responses to eotaxin.

[0089] The inventors have demonstrated for the first time that activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric plexus and neuronal processes of the enteric nervous system in inflammatory bowel disease, and that treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus and neuronal processes of the ENS.

[0090] Accordingly, in a specific embodiment, the invention provides a method of treating and/or preventing a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function, wherein the modulator of an enteric nervous system (ENS)-controlled enteric function is a modulator of CCR3 activity and/or an Eotaxin-1 activity.
[0091] As shown in examples 3, 7 and 15 to 18 administration of a CCR3 antagonist either before or following a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function wherein, the modulator of an enteric nervous system (ENS)-controlled enteric function is CCR3 antagonist SB328437.

[0092] Accordingly, in a specific embodiment, the present invention provides a method of treating and/or preventing a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function wherein, the modulator of an enteric nervous system (ENS)-controlled enteric function is CCR3 antagonist SB328437.

[0093] Enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease. Surprisingly, and for the first time, the inventors have demonstrated that treatment with a CCR3 antagonist before or after induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility, and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0094] Resveratrol is a natural polyphenolic compound with strong antioxidant properties, has neuroprotective activity, partially explaining its ability to be useful in controlling such neurodegenerative diseases as Alzheimer's and Parkinson's (Pallas et al. 2009). Resveratrol is a sirtuin-activating compound; activation of sirtuin-1 (involved in responding to molecular damage and metabolic imbalances) prevents apoptosis in brain neurons (Pallas et al. 2009). Recently sirtuin-1 has been localized in the mouse colon enteric neurons (Lakhan & Kirchgessner, 2011). Resveratrol has minimal or no toxicity on the body systems (Johnson et al. 2011). To date, the neuroprotective activity of resveratrol has not been evaluated in enteric neurons.

[0095] As shown in Examples 11 and 19 and depicted in Figures 13 and 19 herein, the inventors have demonstrated that enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0096] Accordingly, in an embodiment of the present invention the modulator of an enteric nervous system (ENS)-controlled enteric function is resveratrol.
[0097] In a further embodiment, the modulator is a combination of a CCR3 antagonist and resveratrol.

[0098] In some embodiments, therapy by administration of one or more modulators of the present invention is combined with the administration of one or more therapies such as, but not limited to, anti-inflammatory therapies, hormonal therapies, and/or biological therapies/immunotherapies. Prophylactic/therapeutic agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, including post-translationally modified proteins, antibodies etc.; or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules. Prophylactic/therapeutic agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules.

[0099] The modulators of the present invention may be included compositions comprising a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of one or more modulators of the invention and a pharmaceutically acceptable carrier. In a further embodiment, the modulators of the invention further comprise an additional anti-inflammatory agent. Anti-inflammatory agents can be steroidal or non-steroidal, as is known in the art.

[0100] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agent. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject. A first prophylactic or therapeutic agent can be administered prior to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent to a subject. The prophylactic or therapeutic agents are administered to a subject in a sequence and within a time interval such that the agent of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. Any
additional prophylactic or therapeutic agent can be administered in any order with the other additional prophylactic or therapeutic agents.

[0101] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0102] The compositions as used in the present invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0103] Any compound which inhibits the activity of CCR3 may be used in the present invention. Such compounds include inhibitory molecules which bind directly to the CCR3 receptor, antibodies which bind the CCR3 receptor or to the natural ligands of the CCR3 receptor, including eotaxin (CCLI 1), eotaxin-2 (CCL24) and eotaxin-3 (CCL26), RNA, DNA or RNA/DNA aptamers which specifically bind CCR3, eotaxin, eotaxin-2 or eotaxin-3, and siRNA or anti-sense oligonucleotides which inhibit the expression of CCR3, eotaxin, eotaxin-2 or eotaxin-3. Additional compounds which inhibit the activity of CCR3 include inhibitory molecules which specifically bind CCR3, eotaxin, eotaxin-2 or eotaxin-3, including an oligopeptide, small molecule antagonist (e.g., organic molecule having a molecular weight less than 2000, or less than 1000, or less than 500), ribozyme, intrabody or intraceptor. An intrabody refers to an antibody produced intracellularly, for example, a single chain antibody (such as a single chain Fv antibody fragment) expressed inside a cell transformed with an appropriate exogenous nucleic acid. An intraceptor refers to an receptor (such as a receptor for CCR3, eotaxin, eotaxin-2 or eotaxin-3) produced intracellularly, for example, a CCR3, eotaxin, eotaxin-2 or eotaxin-3 receptor expressed inside a cell transformed with an appropriate exogenous nucleic acid.

[0104] In one aspect the CCR3 antagonist is SB328437; (S)-Methyl-2-naphthoylamino-3-(4-nitrophenyl)propionate (also known as SB328437). SB328437 is commercially available, for example from Calbiochem.
In another aspect the CCR3 antagonist is W-56750; [4-(3-aminophenyl)thiazol-2-ylthio]-N-[1-(3,4-dichlorobenzyl)piperidin-4-yl] acetamide. W-56750 is available commercially from Mitsubishi Tanabe Pharma Co.

Numerous "small molecule" inhibitors for the CCR3 receptor have been developed and can be used in the present invention.

In one aspect the CCR3 antagonist is an organic molecule having a molecular weight less than 2000. In another aspect of the invention, the CCR3 antagonist is an organic molecule having a molecular weight less than 1000. In yet another aspect of the invention, the CCR3 antagonist is an organic molecule having a molecular weight less than 500. The CCR3 receptor inhibitors include piperidine derivatives, piperidine amides and 'biperidine compounds such as those described in U.S. Patent Nos. 6,984,651 and 6,903,115, and U.S. published applications 20050176708, 20050182094 and 20050182095; heterocyclic piperidines such as those described in U.S. Patent No. 6,759,411; diphenyl-piperidine derivatives such as those described in U.S. Patent No. 6,566,376; 2,5-substituted pyrimidine derivatives such as those described in U.S. Patent No. 9,684,643; piperizinones such as those described in U.S. Patent No. 6,974,869; bicyclic and tricyclic amines such as those described in U.S. Patent No. 6,960,666; N-ureidoalkyl-piperidines such as those described in U.S. Patent Nos. 6,949,546, 6,919,368, 6,906,066, 6,897,234, 6,875,776, 6,780,857, 6,627,629, 6,527,592 and 6,331,541; bicyclic diamines such as those described in U.S. Patent No. 6,821,964; benzylcycloalkyl amines such as those described in U.S. Patent No. 6,864,380; 2-substituted-4-nitrogen heterocycles such as those described in U.S. Patent No. 6,706,735; ureido derivatives of poly-4-amino-2-carboxy-l-methyl pyrrole compounds; bicyclic and bridged nitrogen heterocycles such as those described in U.S. published application 20050234034; azetidine derivatives such as those described in U.S. published application 20050222218; substituted fused bicyclic amines such as those described in U.S. published application 20050197373; substituted spiro azabicicycles such as those described in U.S. published application 20050197325; piperidine-substituted indoles or heteroderivatives thereof such as those described in U.S. published application 20050153979; piperidinyl and piperazinyl compounds substituted with bicyclo-heterocyclalkyl groups such as those described in U.S. published application 20050090504; arylsulfonamide derivatives such as those described in U.S. published application 20050070582; 1-phenyl-1,2-diaminoethane derivatives such as those described in U.S. published application 20040063779; (N-[(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl)-N'[(2-methyl-2H-tetrazol-5-yl)methyl]urea) (see, e.g., Nakamura et al., Immunol Res., 33:213-222, 2006; the CCR3 antagonist compounds described in Suzuki et al., Biochem. Biophys. Res. Commun., 339:1217-1223, 2006;

[0108] Additional compounds for inhibiting the CCR3 receptor include RNA, DNA or RNA/DNA aptamers directed against CCR3, eotaxin, eotaxin-2 or eotaxin-3. Exemplary methods for making aptamers are described in U.S. Patent Nos. 5,270,163, 5,840,867, 6,180,348 and 6,699,843. Additional compounds for inhibiting the CCR3 receptor include anti-sense oligonucleotides or siRNAs directed against CCR3, eotaxin, eotaxin-2 or eotaxin-3, including the anti-sense oligonucleotides directed against the CCR3 receptor such as that described in U.S. Patent No. 6,822,087. The siRNAs for use in the present invention are designed according to standard methods in the field of RNA interference. Introduction of siRNAs into cells may be by transfection with expression vectors, by transfection with synthetic dsRNA, or by any other appropriate method. Transfection with expression vectors is preferred. The expression vectors which can be used to deliver siRNA according to the invention include retroviral, adenoviral and lentiviral vectors. The expression vector includes a sequence which codes for a portion of the target gene (e.g., CCR3 receptor, eotaxin, eotaxin-2 or eotaxin-3) which is to be silenced. The target gene sequence is designed such that, upon transcription in the transfected host, the target RNA sequence forms a hairpin structure due to the presence of self-complementary bases. Processing within the cell removes the loop resulting in formation of a siRNA duplex. The double stranded RNA sequence should be less than 30 nucleotide bases; preferably the dsRNA sequence is 19-25 bases in length; more preferably the dsRNA sequence is 20 nucleotides in length. The expression vectors may include one or more promoter regions to enhance synthesis of the target gene sequence. Promoters which can be used include CMV promoter, SV40 promoter, promoter of mouse U6 gene, and promoter of human H1 gene. One or more selection markers may be included to facilitate transfection with the expression vector. The selection marker may be included within the expression vector, or may be introduced on a separate genetic element. For example, the bacterial hygromycin B phosphotransferase gene may be used as a selection marker, with cells being grown in the presence of hygromycin to select for those cells transfected with the aforementioned gene. Synthetic dsRNA may also be introduced into cells to provide gene silencing by siRNA. The synthetic dsRNAs are less than 30 base pairs in length. Preferably the synthetic dsRNAs are 19-25 base pairs in length. More preferably the dsRNAs are 19, 20 or 21 base pairs in length, optionally with 2-nucleotide 3’ overhangs. The 3’ overhangs are preferably TT residues.
[0109] Synthetic dsRNAs can be introduced into cells by injection, by complexing with agents such as cationic lipids, by use of a gene gun, or by any other appropriate method. Additional compounds for inhibiting the CCR3 receptor include antibodies which specifically bind the CCR3 receptor, eotaxin, eotaxin-2, or eotaxin-3. Exemplary antibodies which specifically bind and inhibit the CCR3 receptor are described in U.S. Patent Nos. 6,806,061 and 6,207,155, and in U.S. published applications 20050191702, 20050069955, and 20020147312. Exemplary antibodies which specifically bind and inhibit eotaxin and eotaxin-2 are described in U.S. Patent Nos. 6,946,546 and 6,635,251, and in U.S. published applications 20040191255 and 20040014132.

[0110] The antibodies employed in the present invention can be polyclonal or monoclonal, and the term antibody is intended to encompass both polyclonal and monoclonal antibodies. Antibodies of the present invention can be raised against an appropriate immunogen, including proteins or polypeptides of the present invention, such as isolated and/or recombinant mammalian CCR3 receptor, eotaxin, eotaxin-2 or eotaxin-3 protein or portion thereof, or synthetic molecules, such as synthetic peptides.

[0111] Preparation of immunizing antigen, and polyclonal and monoclonal antibody production can be performed using any suitable technique. A variety of methods have been described (see e.g., Kohler et al., Nature, 256: 495-497 (1975) and Eur. J. Immunol. 6: 511-519 (1976); Milstein et al., Nature 266: 550-552 (1977); Koprowski et al., U.S. Pat. No. 4,172,124; Harlow, E. and D. Lane, 1988, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y.); Current Protocols in Molecular Biology, Vol. 2 (Supplement 27, Summer -94), Ausubel, F. M. et al., Eds., (John Wiley & Sons: New York, N. Y.), Chapter 11, (1991)). Generally, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0) with antibody producing cells. The antibody producing cell, preferably those of the spleen or lymph nodes, are obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) are isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired specificity are selected by a suitable assay (e.g., ELISA).

[0112] Single chain antibodies, and chimeric, humanized or. primatized (GDR-grafted) antibodies, as well as chimeric or CDR-grafted single chain antibodies, comprising portions derived from different species, are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic
engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., European Patent No. 0,125,023. Bl; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., European Patent No. 0,120,694 Bl; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S. et al., European Patent No. 0,194,276 Bl; Winter, U.S. Pat. No. 5,225,539; and Winter, European Patent No. 0,239,400 Bl. See also, Newman, R. et al., BioTechnology, 10: 1455-1460 (1992), regarding primatized antibody, and Ladner et al., U.S. Pat. No. 4,946,778 and Bird, R. E. et al., Science, 242: 423-426 (1988)) regarding single chain antibodies.

[0113] In addition, functional fragments of antibodies, including fragments of chimeric, humanized, primatized or single chain antibodies, can also be employed. Functional fragments of foregoing antibodies retain at least one binding function and/or modulation function of the full-length antibody from which they are derived. For example, antibody fragments capable of binding to a mammalian CCR3 receptor, etoxatin, etoxatin-2 or etoxatin-3 or portion thereof, including, but not limited to, Fv, Fab, Fab1 and F(ab').sub.2 fragments are encompassed by the invention. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can generate Fab or F(ab').sub.2 fragments, respectively. Alternatively, antibodies can be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site. For example, a chimeric gene encoding a F(ab').sub.2 heavy chain portion can be designed to include DNA sequences encoding the CH.sub.1 domain and hinge region of the heavy chain.

[0114] The antibodies of the present invention can be used to modulate receptor or ligand function in research and therapeutic applications. For instance, antibodies can act as inhibitors to inhibit (reduce or prevent) (a) binding (e.g., of a ligand, a second inhibitor or a promoter) to the receptor, (b) a receptor signalling, (c) and/or a stimulatory function. Antibodies which act as inhibitors of receptor function can block ligand or promoter binding directly or indirectly (e.g., by causing a conformational change). For example, antibodies can inhibit receptor function by inhibiting binding of a ligand, or by desensitization (with or without inhibition of binding of a ligand).

[0115] Anti-idiotypic antibodies are also provided. Anti-idiotypic antibodies recognize antigenic determinants associated with the antigen-binding site of another antibody. Anti-idiotypic antibodies can be prepared against a second antibody by immunizing an animal of the same species, and preferably of the same strain, as the animal used to produce the second
antibody. See e.g., U.S. Pat. No. 4,699,880. Single chain, and chimeric, humanized or
primatized (CDR-grafted), as well as chimeric or CDR-grafted single chain anti-idiotypic
antibodies can be prepared, and are encompassed by the term anti-idiotypic antibody.
Antibody fragments of such antibodies can also be prepared.

[01 16] One or more inhibitors of CCR3 receptor function, such as those identified as
described herein, can be used to prevent or treat a bowel disorder.

[01 17] The present invention also provides methods of screening for modulators of CCR3
activity:

[01 18] To determine if a candidate molecule is a functional CCR3 antagonist, the effects of
the candidate molecule on a CCR3 function can be examined using methods known in the
art. For example, the effect of a candidate molecule on intracellular calcium mobilization
stimulated by eotaxin, eotaxin-2, or MCP-4 can be monitored. For example, eotaxin, eotaxin-
2, or MCP-4 with IC50 values for inhibition of calcium mobilization can be determined, and
concentration dependence determined.

[01 19] To determine if a candidate molecule is a specific CCR3 antagonist, the effects of the
candidate molecule on inhibition of binding of other receptors can be examined using
methods known in the art. For example, the effect of a candidate molecule on inhibition of the
binding of a number of agonists to their respective cognate 7-TM receptors may be
monitored.

[0120] SB 328437 is highly potent and selective CCR3 receptor antagonist with IC50 of <20
nM. At higher doses SB 328437 also inhibits eotaxin with IC50 of 55nM.

[0121] The effective amount of a composition to be administered can be dependent on any
number of variables, including without limitation, the species, breed, size, height, weight, age,
overall health of the subject, the type of formulation, the dose of radiation received or
anticipated, or the amount of time before or the amount of time elapsed since exposure to
radiation. The appropriate effective amount can be routinely determined by those of skill in
the art using routine optimization techniques, the skilled and informed judgment of the
practitioner, and other factors evident to those skilled in the art. Preferably, a therapeutically
effective dose of the compounds described herein will provide therapeutic benefit without
causing substantial toxicity to the subject.
In one embodiment the modulator is administered at a dose of about 0.01 mg to about 25000 mg per day, 10 mg to about 20000 mg per day, 25 mg to about 15000 mg per day, or about 100 mg to about 2000 mg per day.

In one embodiment the modulator is administered to a human subject at a dose of about 1 mg/kg. This dose relates to a dose of about 5 mg/kg in guinea pigs, calculated according to the method of Regan-Shaw et al. (2007) FASEB Journal 22: 659.

In one embodiment the modulator is administered to a human subject at a dose of about 3 mg/kg. This dose relates to a dose of about 13.75 mg/kg in guinea pigs, calculated according to the method of Regan-Shaw et al. (2007) FASEB Journal 22: 659.

In one embodiment resveratrol is administered to a human subject at a dose of about 0.1 mg/kg. This dose relates to a dose of about 0.5 mg/kg in guinea pigs, calculated according to the method of Regan-Shaw et al. (2007) FASEB Journal 22: 659.

Toxicity and therapeutic efficacy of agents or compounds can be assayed using standard pharmaceutical procedures in a variety of systems and environments, including cell-free environments, cellular environments (e.g., cell culture assays), multicellular environments (e.g., in tissues or other multicellular structures), and/or in vivo (e.g., in experimental animals), e.g., by determining the LD50 (the dose lethal to 50% of the population) and/or the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50.

Data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in the subject. The dosage of such agents or compositions lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized.

According to one preferred embodiment, any of the compositions of the invention may be administered orally or by inhalation as an aerosol or by intravenous, intramuscular, subcutaneous, intraperitoneal, parenteral, transdermal, intravaginal, intranasal, mucosal, sublingual, topical, rectal or subcutaneous administration, or any combination thereof.
[0129] The preparation of pharmaceutical compositions is well known in the art and has been described in many articles and textbooks, see e.g., Remington's Pharmaceutical Sciences, Gennaro A. R. ed., Mack Publishing Co., Easton, PA, 1990, and especially pp. 1521-1712 therein, fully incorporated herein by reference.

[0130] The pharmaceutical composition of the invention can be administered and dosed in accordance with good medical practice.

[0131] The composition of the invention may comprise the active substance in free form and be administered directly to the subject to be treated. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient.

[0132] Formulations include those suitable for oral, nasal, or parenteral (including subcutaneous (s.c), intramuscular (i.m.), intraperitoneal (i.p.), intravenous (i.v.) and intradermal or by inhalation to the lung mucosa) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The nature, availability and sources, and the administration of all such compounds including the effective amounts necessary to produce desirable effects in a subject are well known in the art and need not be further described herein.

[0133] The pharmaceutical compositions of the invention generally comprise a buffering agent, an agent that adjusts the osmolarity thereof, and optionally, one or more pharmaceutically acceptable carriers, excipients and/or additives as known in the art. Supplementary active ingredients can also be incorporated into the compositions. The carrier can be solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[0134] As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic composition is contemplated.
it should be noted that for the method of treatment and prevention provided in the present invention, said therapeutic effective amount, or dosage, is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. In general, dosage is calculated according to body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the composition of the invention in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the composition of the invention is administered in maintenance doses, once or more daily.
EXAMPLES

EXAMPLE 1: Methods

Experimental Design

[0136] Guinea pigs were divided into the following five groups: 1) Control, 2) Sham, 3) TNBS, 4) TNBS + vehicle injection, 5) TNBS treated with CCR3 antagonist (see Table 1). The CCR3 antagonist used is SB-328437 (Merck). SB328437 is an antagonist of eotaxin-1 and/or CCR3, and consequently inhibits eosinophil accumulation. SB328437 was given by a single intraperitoneal injection to the treated group one hour prior to TNBS administration. Segments of the colon were collected 24 hours and 7 days post TNBS administration, the same method applied to TBNS + vehicle animals. Control animals did not receive any treatment. Sham animals received sterile saline instead of TNBS and no SB328437 treatment. Experiments involved 1) quantification of EDN and evaluation of proximity to enteric neurons of myenteric plexus (immunohistochemistry), 2) quantification of eosinophil presence within the lamina propria (histology); 3) quantification of enteric neurons in the myenteric plexus (immunohistochemistry) and 3) examine changes in function of the gut (motility).

Table 1: Experimental design

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Control</th>
<th>Sham</th>
<th>TNBS</th>
<th>TNBS + vehicle</th>
<th>TNBS + CCR3 low dose</th>
<th>TNBS + CCR3 high dose</th>
<th>CCR3 + TNBS</th>
<th>TNBS + Resveratrol</th>
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<tr>
<td>Gastrointestinal motility</td>
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<td>(n=4)</td>
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<td>(n=3)</td>
<td>(n=3)</td>
<td>(n=3)</td>
<td>(n=3)</td>
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<td>Eosinophil accumulation</td>
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<td>(n=3)</td>
<td>(n=3)</td>
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<tr>
<td>Correlation between eosinophil derived neurotoxin and enteric neuronal damage</td>
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</table>
Animal Surgical Procedures

[Hartley Guinea pigs (140-280g) of either sex obtained from the Institute of Medical and Veterinary Science (Adelaide) were housed at Melbourne University Animal facility. Rooms were maintained at approximately 20°C at all times with 12 hour light/dark cycle. Animals were given three days acclimatization period after arrival and allowed food and water at libitum. On treatment days animals were anesthetized with isoflurane and inflammation in the colon was induced with 0.3ml of TNBS (30µg/kg bodyweight) in 30% ethanol for TNBS groups or normal Krebs solution (composition, mM KCl 4.6, NaCl2 118, MgSO4 1.2, NaHC03 25, NaH2P04 1, CaCl2 2.5, D-glucose 11) for sham animals delivered through a polyethylene catheter inserted rectally 6cm proximal to the anus. Animals were given CCR3 antagonist SB-328437 at low (5mg/kg) and high doses (13.75 mg/kg) dissolved in DMSO and 1x phosphate buffered saline (PBS) by intraperitoneal (IP) injection (TNBS + CCR3 group) or injection of vehicle solution containing DMSO and 1x PBS (TNBS + vehicle group). IP injections were given one hour prior to TNBS administration for both groups.

Tissue collection and preparation

[Tissues were collected 24 hours and 7 days after treatment. Animals were killed by the method of being stunned by a blow to the head then exsanguinated by severance of their carotid arteries and spinal cord. This procedure complies with guidelines of the National Health and Medical Research Council of Australia and was approved by the Victoria University Animal Experimentation Ethics Committee. The abdominal cavity was opened and an 8-10cm segment of the colon cut just proximal to the anus was collected with the oral end being marked by a small pin and placed into Krebs solution infused with carbogen (95% oxygen and 5% carbon dioxide). Tissue was then used for motility, histology and immunohistochemistry experiments.

Immuno#histochemistry

[A small section of the colon (~1cm) was taken and cut along its mesenteric border in Krebs containing nicardipine (3µM) and pinned out flat in a silicon lined Petri dish. Tissue was fixed overnight at 4°C in Zamboni’s fixative (containing 2% formaldehyde and 0.2% picric acid). Tissue was then washed with DMSO 3x10minut.es before being washed with PBS 3x10minut.es prior to being placed for storage in PBS containing 0.1% sodium azide. Before processing tissue was frozen in OCT blocks with the use of liquid nitrogen and cut on the cryostat in 30µm sections and mounted onto slides. Cryostat sections were washed in PBS+triton 2x5mins and incubated with 10% normal Donkey serum 1 hour before being labelled with Eosinophil-derived Neurotoxin mouse anti- human polyclonal primary antibody]
(Abeam) 1:500 (overnight) and Alexa Flour 594 (excitation wavelength 559nm) donkey anti-mouse secondary antibody 1:200 (2 hour incubation) (Abeam). The sections were counter-labelled with β-Tubulin rabbit polyclonal primary antibody (Abeam) (overnight) 1:1000 and Alexa Fluor 488 (excitation wavelength 473nm) donkey anti-rabbit secondary antibody 1:200 (Abeam) (2 hour incubation). Sections were then coverslipped with DAKO mounting media and examined by use of Olympus Fluoview FV1000 confocal laser scanning microscope. Three dimensional Z-sections with a step size of 1.5 μm for evidence of EDN being close to enteric neurons.

**Quantification of EDN accumulation**

[0140] Cross sections were observed under a fluorescent microscope (Olympus BX53, Olympus, Australia). Random sections within myenteric ganglia were counted using an eyepiece containing 0.5cm grid. Four random sections of each cross section were counted. This was repeated using 4 cross sections from each animal cut at least 100 μm apart to minimise chance of repeated cell counting. Data were averaged per 1 cm² before being converted to value per cm².

**Histology**

[0141] Standard Haematoxylin and Eosin histological staining was used to identify and quantify the number of eosinophils in cross sections of the colon. A 1 cm section of the colon was taken and cut along its mesenteric border in Krebs containing nicardipine (3 μM) and pinned out flat in a silicon lined Petri dish. Tissue was fixed overnight at 4oC in Zamboni’s fixative. Tissue was then washed with DMSO and 1xPBS 3x10 minutes before being placed for storage in PBS containing 0.1% sodium Azide. Before being processed tissue was impregnated with paraffin and embedded in paraffin blocks. Tissue was cut using microtome at 5 μm section thickness and placed in a water bath at 45oC and mounted onto slides. Slides were placed into a 60o oven for 30 minutes prior to staining. Sections were deparaffinised by use of 2x4 min wash in histolene and then dehydrated by 2 mins in 100% ethanol, 2 mins in 90% ethanol, and 2 mins in 70% ethanol. Tissue was then washed in tap water before being stained in haematoxylin 4 minutes, tissue was washed again 30-60 seconds then allowed to develop blue colour in Scotts tap water 1 min. The tissue was washed again in tap water 3 min and then counterstained with alcoholic eosin 4 minutes before being washed again in tap water for 1 minute. The tissue was then dehydrated in 100% ethanol 2x 30 seconds and cleared using histolene 2x4 minutes. Eosinophils can be recognized as binuclear cells with cytoplasm stained pink by eosin and nucleus stained as purple/blue by haematoxylin (Pontell et al., 2009).
Motility

[0142] Tissue was placed into an organ bath containing approximately 25ml of heated Krebs bubbled with carbogen gas and maintained at 37°C. Krebs solution was continually superfused through the organ bath at a flow rate of 6ml min-1 via means of inflow reservoirs and a vacuum pump. The tissue was allowed to pass all pellets spontaneously before being cannulated at both oral and anal ends and secured with nylon thread. The cannula at the oral end was attached to a two way stopcock reservoir containing carbogenated Krebs solution which feeds directly into the lumen. The height of this reservoir was vertically enabled to be adjusted to increase luminal pressure this was performed in 1cm increments. The anal end cannulae were attached to a three-way vertical stopcock to enable outflow pressure to be measured and to a draining line to allow for flushing of luminal contents when necessary.

[0143] Once the 5-6cm colon segment had been cannulated and approximately equal pressures at both the oral and anal end had been established a series of 3-5x10 minutes control with luminal reservoir height =0 (i.e. basal pressure) video recordings were taken. After the control recordings had been done the luminal reservoir height was increased by 1cm increments to record a further 3x10 minute videos. Increasing the height of the luminal reservoirs increases the pressure at which Krebs is delivered to the lumen. Increased pressure causes the intestine to dilate more than normal and consequently stimulates more frequent and forceful contractions. Recordings were performed with the use of a Logitech Quickcam Pro video camera set at 6-7cm above the organ bath via live stream recording using Logitech software and converted into avi files in a program called Virtual dub. The avi files were converted into spatiotemporal maps by the use of edge-detection software developed by Bornstein's lab (Melbourne University). The software analyzes changes in gut width over time and converts the data into a grey scale spatiotemporal map where white is used to show the smallest tissue widths (contraction) and black represents the largest widths recorded (dilation). Analysis of spatiotemporal maps yields quantitative data about how many contractions occurred throughout the recording, the type of contraction, direction of origin and the speed of propagation (Gwynne et al., 2004)

Example 2: Analysis of Spatiotemporal Maps

[0144] Speed of propagation is measured by taking the gradient of the contraction as pictured above the value is given in mm/sec. The speed of each whole length contraction per 10
minute recording was measured and averaged for final results. Figure 1 shows analysis of speed propagation of spatiotemporal maps of gut width.

Quiescence

Analysis of quiescence (time interval between contractions) and amplitude of contractions required 3 cross sections to be taken 10mm apart and evaluated. Matlab enables cross sections to be converted into graphical form for evaluation. Quiescence (sec) was determined by taking the time values at the bottom of the contraction peaks and subtracting them from one another to determine the difference. The final values were averaged and used for concluding results. Figure 2 shows analysis of quiescence (time interval between contractions) and amplitude of contractions of spatiotemporal maps of gut width. Figure 3 shows determination of quiescence (time interval between contractions) of gut contractions.

Amplitude

The values for amplitude (mm) were obtained by taking the values from the top and bottom of the contraction peaks and subtracting them from one another appropriately to determine the difference i.e. amplitude. Figure 4 shows determination of amplitude of gut contractions.

Other variables

Contractions had to propagate the entire length of the tissue to be classed as a whole length contraction (WLC). If contractions did not satisfy this requirement but did however propagate past the midpoint of the tissue they were classified as half length contractions. The direction of their origin was also taken into consideration and these types of contractions were classed as either oral half length contractions (OHLC) or anal half length contractions (AHLC). Any contraction that occurred but did not propagate past the midpoint of the tissue was classed as non propagating contractions. Again the direction of their origin was used to differentiate these contractions, they could be either oral non propagating (ONP) or anal non propagating contractions (ANP).

Statistical Analysis

All data were assessed using one-way ANOVA with Tukey’s post hoc test. Analyses were performed using Graph Pad Prism statistical program. Graphs were obtained from
Graph Pad Prism output. All data are presented as mean ± standard error of the mean (SEM). P values less than 0.05 were considered significant.

**EXAMPLE 3**: Activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric plexus and neuronal processes of the enteric nervous system in inflammatory bowel disease, and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus and neuronal processes of the enteric nervous system.

Cross sections taken from every animal show the presence of EDN with the use of immunohistochemical analysis (Figure 5).

The number of EDN cells varied between groups (see Table 2). The largest presence of EDN was observed in the TNBS + vehicle group. This group was significantly different ($P <0.0001$) to all other groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>TNBS (D1)</th>
<th>CCR3 low dose + TNBS (D1)</th>
<th>TNBS (D7)</th>
<th>CCR3 low dose + TNBS (D7)</th>
<th>CCR3 high dose + TNBS (D7)</th>
<th>Resveratrol + TNBS (D7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.61</td>
<td>3.22</td>
<td>52.1*</td>
<td>6.22</td>
<td>68.4*</td>
<td>27.0</td>
<td>2.0</td>
<td>42</td>
</tr>
<tr>
<td>SEM</td>
<td>1.4</td>
<td>0.6</td>
<td>3.5</td>
<td>2.9</td>
<td>10.3</td>
<td>2.7</td>
<td>1.5</td>
<td>9.02</td>
</tr>
</tbody>
</table>

*** $P<0.001$ significantly different from all other groups except Resveratrol + TNBS.

The TNBS group was also significantly different from all other groups ($P<0.001$). Interestingly there was no significant difference between the control group and the sham surgery group. Furthermore there was no significant difference between control; sham or TNBS + CCR3 groups (Table 2).
Treatment with CCR3 antagonist ("TNBS+CCR3") was significantly different from TNBS alone and TNBS plus vehicle, indicating that SB-328437 inhibits EDN accumulation in the myenteric plexus in inflammatory bowel disease.

Figure 5 shows activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric plexus and neuronal processes of the enteric nervous system in inflammatory bowel disease, and that administration of the CCR3 antagonist SB 328437 before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus and neuronal processes of the enteric nervous system.

EXAMPLE 4: Inflammation induced damage to myenteric ganglia and neuronal processes in inflammatory bowel disease, and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents damage to myenteric ganglia and neuronal processes.

Increase in the number of EDN positive cells correlates with the extent of damage incurred to myenteric ganglia (see Figure 6), which shows administration of the CCR3 antagonist SB 328437 prevents inflammation induced damage to myenteric ganglia and neuronal processes.

EXAMPLE 5: Inflammation induced damage to neuronal projections within the mucosa, and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents inflammation induced damage to neuronal projections within the mucosa.

Increase in the number of EDN positive cells correlates with the extent of damage incurred to neuronal processes projecting to the mucosa (see Figure 7).

Figure 7 shows fluorescent images x20 magnification showing inflammation induced damage to neuronal projections within the mucosa stained with β-tubulin. (A) shows normal appearance of neuronal processes within mucosa in control tissue (B) shows partial loss of neuronal processes of following TNBS treatment, 24 hours after TNBS administration (C) shows almost complete loss of neuronal projections following TNBS + vehicle treatment (D) shows reduced loss of neuronal projections following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating administration of the CCR3 antagonist SB 328437 before induction of inflammatory bowel
EXAMPLE 6: Inflammation induced changes to histological structure of guinea pig colon, and treatment with CCR3 antagonist before induction of inflammatory bowel disease reduces transmural inflammation and mucosal insult.

[0157] Administration of CCR3 antagonist effectively reduced the severity of the damage to the ganglia and axons caused by inflammation (Figures 6D and 7D).

[0158] Histological assessment of colon sections (5 µm) showed obvious changes of the colon wall due to transmural inflammation induced by TNBS (Figure 8B). Insult to mucosa is also present in TNBS animals. Similar damage can be seen in the tissue from TNBS + vehicle group (Figure 8C).

[0159] Administration of the CCR3 antagonist SB 328437 resulted in markedly reduced severity of transmural inflammation and mucosal insult (Figure 8D).

[0160] This data demonstrates reduced transmural inflammation and mucosonal insult following administration of the CCR3 antagonist SB 328437 prior to induction of inflammatory bowel disease using TNBS treatment, demonstrating administration of CCR3 antagonist before induction of inflammatory bowel disease reduces transmural inflammation and mucosonal insult.

EXAMPLE 7: Quantification of EDN-positive cell accumulation within the myenteric plexus at 1 and 7 days following induction of inflammatory bowel disease using TNBS treatment, and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus of the enteric nervous system.

[0161] Quantification of EDN positive cells within the myenteric plexus per cm² is shown in Figure 9 for 1 and 7 days post TNBS treatment ("1 D" and "7D", respectively) in animals following administration of TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). This data shows that administration of the CCR3 antagonist SB 328437 prior to induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric plexus of the enteric nervous system.
EXAMPLE 8: Enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility.

[0162] Figure 10 shows enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and that administration of CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. The average number of contractions per minute are shown in Figure 10 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following administration of TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). This data shows that administration of the CCR3 antagonist SB 328437 before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. The prevention of reduction of enteric nervous system controlled intestinal motility was observed at both Day 1 and Day 7.

EXAMPLE 9: Enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility at different levels of intraluminal pressure.

[0163] Functional assessment of colon tissue was performed on every animal within every group with the use of video imaging experiments. Parameters used to assess the function of the tissue were speed, quiescence and amplitude. Also the number of different types of contractions were quantified and compared to control animals. All of these parameters were assessed at 4 different levels of intraluminal pressure: basal, 1 cm above basal, 2 cm above basal and 3 cm above basal.

[0164] The average number of contractions per minute are shown in Figure 11 at 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following administration of TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("TNBS + CCR3"). The data is shown for basal pressure ("base"), 1 cm above basal pressure (+1"), 2 cm above basal pressure (+2"), and 3 cm above basal pressure (+3"). This data shows that administration of the CCR3
antagonist SB 328437 before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. The prevention of reduction of enteric nervous system controlled intestinal motility was observed at both Day 1 and Day 7, and for each intraluminal pressure.

EXAMPLE 10: Enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility at different levels of intraluminal pressure.

[0165] The length of contractions per minute are shown in Figure 12 for 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in animals following administration of TNBS only ("TNBS"), and following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("CCR3"). The data is shown for 1 cm above basal pressure ("1"), 2 cm above basal pressure ("2"), 3 cm above basal pressure ("3"), and 4 cm above basal pressure ("4"). This data shows that administration of the CCR3 antagonist SB 328437 before induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. The prevention of reduction of enteric nervous system controlled intestinal motility was observed at both Day 1 and Day 7, and for each intraluminal pressure.

EXAMPLE 11: Enteric nervous system controlled intestinal motility is reduced following induction of inflammatory bowel disease, treatment with CCR3 antagonist before or after induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility, and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0166] SB 328437 is highly potent and selective CCR3 receptor antagonist with IC50 of <20 nM; at higher doses SB 328437 also inhibits eotaxin with IC50 of 55nM.

[0167] The effects of 'low' (5mg/kg) and 'high' doses (13.75 mg/kg) of SB 328437 were determined in experiments with i.p. injections of SB 328437 (volume 100-200ul calculated according to the animals' body weight) one hour prior to induction of inflammation by TNBS.

[0168] The low dose of the CCR3 antagonist SB 328437 was used to compare the effects of
SB 328437 given 1h prior to and 1h after TNBS administration. Segments of the colon were collected at Days 1 and 7 after TNBS administration for immunohistochemical studies (n=3-6) and motility experiments (n=3-7).

[0169] Resveratrol (500 ug/kg/d, Sigma, Australia) was injected i.p. (volume 100-200uL calculated according to the animals' body weight) one day prior induction of inflammation by trinitrobenzene sulfonic acid (TNBS) followed by every day i.p. injections. TNBS (30 mg/kg; Sigma, Australia) in 30% ethanol (total volume = 100 uL) was administered into the lumen of the colon through a polyethylene catheter (external diameter = 1.2mm) inserted via the rectum 7 cm proximal to the anus. Animals were killed at Day 7 post-TNBS and segments of the colon were collected for immunohistochemical and motility studies.

[0170] The total number of the colon whole length contractions induced by 3mm of intraluminal pressure per minute are shown in Figure 13 for 1 and 7 days post TNBS treatment ("Day1" and "Day7", respectively) in sham treated animals ("Shams"), following administration of TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("PreTNBS"), administration of CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment ("Post TNBS"), and administration of Resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Resveratrol"). This data shows that administration of the CCR3 antagonist SB 328437 prior to or following, or administration of resveratrol prior to, induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility.

[0171] This data also shows administration of high dose of the CCR3 antagonist SB 328437 prevents reduction of enteric nervous system controlled intestinal motility.

**EXAMPLE 14:** Survival of enteric neurons in the myenteric plexus is reduced following induction of inflammatory bowel disease, and treatment with CCR3 antagonist or Resveratrol after induction of inflammatory bowel disease promotes survival of enteric neurons in the myenteric plexus.

[0172] The number of enteric neurons are shown in Figure 14 for 1 and 7 days post TNBS treatment ("1D" and "7D", respectively) in sham treated animals ("Sham"), following administration of TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS, and administration of Resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Resveratrol"). This data
shows that administration of the CCR3 antagonist SB 328437 or resveratrol prior to induction of inflammatory bowel disease promotes survival of enteric neurons in the myenteric plexus. This data also shows administration of a high dose of CCR3 antagonist promotes survival of enteric neurons in the myenteric plexus.

EXAMPLE 15: Activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present within the myenteric ganglia of the enteric nervous system in inflammatory bowel disease, and treatment with CCR3 antagonist before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric ganglia.

[0173] Figure 15 shows wholemount preparations x20 and x 40 magnification showing EDN-positive cell populations (red) within the myenteric ganglia labelled with anti-B-Tubulin antibody (green) attached to the longitudinal muscles. EDN-positive cells labelled with anti-EDN antibody (red) found at the level of myenteric plexus. Significant accumulation of EDN-positive cells following TNBS treatment are shown at X20 and x40 magnification, and low levels of EDN-positive cells similar to following administration of the CCR3 antagonist SB 328437 prior to induction of inflammatory bowel disease using TNBS treatment. This demonstrates administration of the CCR3 antagonist SB 328437 before induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the myenteric ganglia of the enteric nervous system.

EXAMPLE 16: Activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present the colon in inflammatory bowel disease, and treatment with high or low doses of CCR3 antagonist before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0174] Figure 16 shows immunohistochemical analysis of the number of EDN-positive cells in cross sections of the guinea-pig colon from the animals treated with CCR3 antagonist and Resveratrol. EDN-positive cells were labelled using anti-EDN antibody (red) and neuronal processes were labelled with anti-B-Tubulin antibody (green). This data shows that activated (Eosinophil derived neurotoxin (EDN)-positive) eosinophils are present the colon in inflammatory bowel disease, and that administration of high or low doses of the CCR3 antagonist SB 328437 prior to or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon. This data also demonstrates
resveratrol had no significant effect on the number of eosinophil-derived neurotoxin (EDN)-positive cells in the cross sections of the colon.

EXAMPLE 17: EDN-positive cell accumulation within the colon at day 1 following induction of inflammatory bowel disease using TNBS treatment, and treatment with CCR3 antagonist before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0175] Quantification of EDN positive cells within the colon per cm\(^2\) at 1 day post TNBS treatment (“1D”) is shown in Figure 17 in animals following administration of TNBS only (“TNBS”), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment (“CCR3 + TNBS”), and following administration of CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment (“TNBS + CCR3”). This data shows that administration of the CCR3 antagonist SB 328437 before or following induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

EXAMPLE 18: EDN-positive cell accumulation within the colon at day 7 following induction of inflammatory bowel disease using TNBS treatment, and treatment with high or low dose CCR3 antagonist prior to induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon.

[0176] Figure 18 shows quantification of EDN positive cells within the colon per cm\(^2\) at day 7 post TNBS treatment (“7D”) in animals following administration of TNBS only (“TNBS”), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment (“CCR3 + TNBS”). This data shows that administration of the CCR3 antagonist SB 328437 prior to induction of inflammatory bowel disease prevents accumulation of activated eosinophils within the colon, and that resveratrol had no significant effect on the number of eosinophil-derived neurotoxin (EDN)-positive cells in the cross sections of the colon. This data also shows administration of a high dose of the CCR3 antagonist SB 328437 prevents accumulation of activated eosinophils within the colon.

EXAMPLE 19: Enteric nervous system controlled intestinal motility is reduced
following induction of inflammatory bowel disease, treatment with CCR3 antagonist before or after induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility, and treatment with resveratrol prevents reduction of enteric nervous system controlled intestinal motility.

[0177] The rate of contractions per minute are shown in Figure 19 for 1 and 7 days post TNBS treatment ("D1" and "D7", respectively) in sham treated animals ("Sham"), following administration of TNBS only ("TNBS"), following administration of CCR3 antagonist prior to induction of inflammatory bowel disease using TNBS treatment ("Pre"), administration of CCR3 antagonist following induction of inflammatory bowel disease using TNBS treatment ("Post"), and administration of resveratrol prior to induction of inflammatory bowel disease using TNBS treatment ("Res"). This data shows that administration of the CCR3 antagonist SB 328437 prior to or following, or administration of resveratrol prior to, induction of inflammatory bowel disease prevents reduction of enteric nervous system controlled intestinal motility. This data also shows administration of a high dose of the CCR3 antagonist SB 328437 prevents reduction of enteric nervous system controlled intestinal motility.
CLAIMS

1. A method of treating and/or preventing a bowel disorder comprising administering to a subject in need thereof a therapeutically effective amount of a modulator of an enteric nervous system (ENS)-controlled enteric function.

2. A method according to claim 1 wherein the bowel disorder is inflammatory bowel disease or post-inflammatory irritable bowel syndrome.

3. A method according to claim 1 or claim 2 wherein the modulator of an ENS-controlled enteric function is a modulator of CCR3 activity and/or eotaxin-1 activity.

4. A method according to claim 3 wherein the modulator is a CCR3 antagonist and/or an eotaxin-1 antagonist.

5. A method according to claim 4 wherein the modulator decreases accumulation of EDN in the gastrointestinal tract of the subject.

6. A method according to claim 5 wherein the modulator of an ENS-controlled enteric function decreases accumulation of EDN in the myenteric plexus of the subject.

7. A method according to any one of claims 1 to 6 wherein the modulator is SB-328437.

8. A method according to claim 7 wherein the modulator is administered at a dose sufficient to antagonise CCR3 activity.

9. A method according to claim 7 wherein the modulator is administered at a dose sufficient to antagonise CCR3 activity and eotaxin-1 activity.

10. A method according to claim 1 or claim 2 wherein the modulator of an ENS-controlled enteric function is an antioxidant.

11. A method according to claim 10 wherein the modulator of an ENS-controlled enteric function is resveratrol.

12. A method according to claim 8 wherein the modulator is administered at a dose of at least 1 mg/kg.
13. A method according to claim 9 wherein the modulator is administered at a dose of at least 3 mg/kg

14. A method according to claim 10 wherein the modulator is administered at a dose of at least 0.1 mg/kg/d

15. A composition comprising a modulator of an ENS-controlled enteric function when used for the treatment and/or prevention of a bowel disorder.

16. A composition according to claim 15, wherein the modulator of an ENS-controlled enteric function is a CCR3 antagonist and/or an eotaxin-1 antagonist.

17. A composition according to claim 16 wherein the modulator of an ENS-controlled enteric function is SB-328437.

18. A composition according to claim 15 wherein the modulator of an ENS-controlled enteric function is resveratrol.

19. Use of a modulator of ENS-controlled enteric function in the preparation of a medicament for the prevention and/or treatment of a bowel disorder.

20. A use according to claim 19, wherein the modulator of an ENS-controlled enteric function is a CCR3 antagonist and/or an eotaxin-1 antagonist.

21. A use according to claim 20 wherein the modulator of an ENS-controlled enteric function is SB-328437.

22. A use according to claim 19 wherein the modulator of an ENS-controlled enteric function is resveratrol.
Figure 1

G:\Lauren\090611\Summaries\le090611.con3-edited_interp.su2

Time (secs)
67 133 200 267 333 400 467 533 600

Gut Length (mm)
3.2 3 2.8 2.6 2.4 2.2 2 1.8 1.6 1.4 1.2

Set Colorbar Scale  Minimum Value: 1.0156  Maximum Value: 3.3516  Change

PCT/AU2012/001414
Figure 2

G:\Lauren\090611\Summaries\le090611.con3-edited_interp.su2

Set Colorbar Scale

Minimum Value: 1.0156
Maximum Value: 3.3516
Change
Figure 9

Number EDN per 0.5 cm²

- Control
- TNBS (1D)
- TNBS+CCR3 (1D)
- TNBS (7D)
- TNBS+CCR3 (7D)
Figure 10

Average number of contractions per minute

Ctrl (n=5)  TNBS-D1 (n=4)  TNBS+CCR3-D1 (n=5)  TNBS-D7 (n=5)  TNBS+CCR3-D7 (n=7)

Pressure

Significance levels: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001
Figure 12

![Bar chart showing length of contraction vs pressure level with different groups: Ctrl, TNBSd1, TNBS CCR3 d1, TNBSD7, CCR3 D7.](chart_image)

- **Ctrl**
- **TNBSd1**
- **TNBS CCR3 d1**
- **TNBSD7**
- **CCR3 D7**
Figure 13

Total number of contractions

Number of contractions per minute

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shams N=2</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Day1-TNBS N=4</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Day7-TNBS N=5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Day1-SB 328437 High Dose PreTNBS N=3</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Day7-SB 328437 Low Dose PreTNBS N=7</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Day1-SB 328437 Low dose PostTNBS N=4</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Day7-TNBS Resveratrol N=3</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

*Statistically significant difference
Figure 14

Number of neurons

- Sham
- TNBS (7D)
- Resveratrol+TNBS (7D)
- CCR3+TNBS_high(7D)
- TNBS+CCR3_low(7D)
Figure 18

Number of EDN-positive cells

- TNBS (7D)
- CCR3+TNBS_low (7D)
- CCR3+TNBS_high (7D)
- Resveratrol+TNBS (7D)
Figure 19

The graph shows the rate of contractions per minute for different groups:

- Sham (n=5)
- TNBS D1 (n=4)
- TNBS D7 (n=7)
- High dose Pre D1 (n=3)
- Low dose Pre D7 (n=7)
- Low dose Post D1 (n=4)
- Res TNBS D7 (n=3)

The graph includes error bars indicating variability. The significance levels are indicated by asterisks: * (p < 0.05), ** (p < 0.01), and *** (p < 0.001).
A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/06 (2006.01)  A61K 31/05 (2006.01)  A61P 1/00 (2006.01)

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

B. FIELDS SEARCHED

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)

WPI, MEDLINE, EPODOC, CAPPLUS - Keywords; bowel, intestinal, enteric, gastrointestinal, ibs, ibd, colitis, celiac, crohns, modulate, antagonist, antagonise, inhibit, inhibited, CCR3, chemokine receptor type 3, CCLI, CCL11, EOTAXIN, SB-328437, 247580-43-4, W-56750, resveratol

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X Further documents are listed in the continuation of Box C  
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Date of the actual completion of the international search

14 December 2012

Date of mailing of the international search report

14 December 2012

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
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Authorised officer

Michael Burn
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. 0262832341

Form PCT/ISA/210 (fifth sheet) (July 2009)
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End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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