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Brain-Gut Interactions in Inflammatory Bowel Diseases

Bruno L. Bonaz^{1,2} and Charles N. Bernstein³

(1) Stress et Interactions Neuro-Digestives (SIND), Grenoble Institut des Neurosciences (GIN), Centre de Recherche INSERM 836 UJF-CEA-CHU, Grenoble F-38043, France.

(2) Clinique Universitaire d'Hépatogastroentérologie, CHU Grenoble, Grenoble F-38043, France.

(3) Section of Gastroenterology, Department of Internal Medicine, University of Manitoba IBD Clinical and Research Centre, University of Manitoba, Winnipeg, Manitoba, Canada.

Corresponding author: Pr Bruno Bonaz, Stress et Interactions Neuro-Digestives (SIND), Grenoble Institut des Neurosciences (GIN), Centre de Recherche INSERM 836 UJF-CEA-CHU, et Clinique Universitaire d'Hépatogastroentérologie, CHU Grenoble, BP 217, F-38043, Cedex 09, France. Tel.: +33 4 76765597; fax: +33 4 76765297. E-mail address: BBonaz@chu-grenoble.fr

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Abstract

Psycho-neuro-endocrine-immune modulation through the brain-gut axis likely has a key role in the pathogenesis of inflammatory bowel diseases (IBD). The brain-gut axis involves interactions between the neural components including; a) the autonomic nervous system, b) the central nervous system c) the stress system (hypothalamic-pituitary adrenal axis, HPA) and d) the (i.e. gastro-intestinal) corticotropin-releasing factor system and e) the intestinal response (including the intestinal barrier, the luminal microbiota and the intestinal immune response). Animal models suggest that the cholinergic anti-inflammatory pathway through an anti-TNF effect of the efferent vagus nerve could be a therapeutic target in IBD either through a pharmacological, nutritional or neurostimulating approach. In addition, the psychophysiological vulnerability of IBD patients, secondary to the potential presence of any of mood disorders, distress, increased perceived stress or maladaptive coping strategies underscores the psychological needs of IBD patients. Clinicians need to address these issues with patients since there is emerging evidence that stress or other negative psychological attributes may impact on disease course. Future research may include exploration of markers of brain-gut interactions, including serum/salivary cortisol (as a marker of the HPA axis), heart rate variability (as a marker of the sympatho-vagal balance) or brain imaging studies. The widespread use and potential impact of complementary and alternative medicine, and positive response to placebo (in clinical trials) is further evidence that exploring other psycho-interventions may be important therapeutic adjuncts to the conventional therapeutic approach in IBD.

Introduction

Over the last decade analyses of the pathobiology of irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) have intersected across several domains despite having very distinct etiologies. In recent years there has been heightened interest in exploring the role of inflammation and the gut microbiome in IBS (1); the former a critical underpinning of IBD and the latter a potentially important driver of the aberrant immune response in IBD (2). While alterations in the brain-gut axis have been considered a pillar of the modern view of the pathobiology of IBS (3), there has been increasing interest in the brain-gut axis as it pertains to IBD. Why should the brain-gut axis be solely in the domain of IBS? These are conditions that share many similar symptoms. For the practicing gastroenterologist a common diagnostic dilemma is discerning whether a patient's symptoms are from IBS, IBD or whether a patient harbors both conditions (4). In understanding the pathogenesis of IBD it is important to not only explore the peripheral mediators of inflammation (the cellular elements and their byproducts) but also the drivers of the immunoinflammatory response. Recently, in IBD, the gut microbiome has garnered increasing attention as an integral player in this process (2). However, psycho-neuroimmune modulation may be the platform that serves to interface the human experience, the state of mind, the gut microbiome and the immune response that ultimately drives the phenotypic expression of IBD, which to date has been the focus of IBD therapy. A biopsychosocial understanding of illness describes clinical outcome and disease exacerbation as influencing and strongly influenced by both biological and psychosocial factors (5-7).

In this review we will explore the supporting evidence in animal models for the importance of the psychoneurological basis of intestinal inflammation; and the clinical evidence that these mechanisms impact on disease course; and where they could potentially be harnessed therapeutically.

Neuro-anatomical basis of the brain-gut axis

The brain and the gut communicate through the autonomic nervous system (ANS) and the circumventricular organs (8) both in physiological and pathological conditions. The ANS, represented by the parasympathetic and sympathetic nervous systems (SNS), includes the vagus nerves (VN), the sacral parasympathetic pelvic nerves, and the splanchnic nerves. These are mixed nerves containing afferent fibers (90% for the VN and 50% for sympathetic nerves) and efferent fibers facilitating neurotransmission between the gut and the central nervous system (CNS, brain and spinal cord). The VN typically transmits information to the CNS regarding luminal osmolarity, carbohydrate levels, mechanical distortion of the mucosa, presence of cytostatic drugs and bacterial products while sympathetic afferents classically transmit visceral pain.

Visceral information transmitted through ANS afferents reaches the CNS at 2 different levels: *i)* the nucleus tractus solitarius (NTS), located in the medulla oblongata, receiving VN afferents, and *ii)* the thoraco-lumbar and sacral spinal cord receiving splanchnic and pelvic nerve afferents respectively. In addition, the circumventricular organs, located outside the blood-brain barrier, are sensitive to the vascular content (e.g. circulating interleukins) and modulate the activity of neighboring neurons that in turn stimulate the hypothalamic-pituitary-adrenal (HPA) axis, thereby suppressing mucosal inflammation via glucocorticoids (Figure 1). After reaching the CNS, visceral information is integrated in the central ANS composed of brain regions involved in the autonomic, endocrine, motor, and behavioral responses essential for survival (9). Output of the central ANS is directly linked through many positive and negative feedback loops governing both sympathetic and parasympathetic outputs on peripheral organs including the immune system. Much of these reflex loops is unconscious or becomes conscious in pathological conditions.

Animal studies

Stress effect on the GI tract:

Stress and the CRFergic system: Stress is the response of the organism to a solicitation of the environment (10). The reaction of stress is physiologic but may become pathologic in the case of an imbalance between the capacities of adaptation and the requirement of the environment, leading to functional, metabolic, and even lesional disorders (11). The HPA axis is the classical pathway through which stress induces an adaptation. Corticotropin-releasing factor (CRF), the principal neuromediator of stress, directly administered into the brain reproduces the overall endocrine, behavioral, autonomic, and visceral changes induced by stress in experimental animals (11,12,13). CRF and CRF-related peptides including urocortins (Ucn1,2,3) were first described in the CNS but are also present in the periphery; they exert their biological actions on target cells through 2 receptors: CRF1 and CRF2 (14).

Stress-induced brain-gut perturbations: Stress induces modifications of motility, secretion, visceral sensitivity, intestinal permeability, and local inflammatory responses in the GI tract (15,16). Stress is involved in the initiation and relapse of experimental colitis (17,18). Stress may play a deleterious role in IBD through 8 main pathways (Figure 2):

1) *Activation of mast cells and the SNS:* Mast cells of the intestinal mucosa serve as end effectors of the brain-gut axis and release several mediators, cytokines, and chemokines that can profoundly affect GI physiology under stress conditions by inducing intestinal hyperpermeability and activation of the mucosal immune function (15,19). Mast cells are in close contact with sympathetic and VN terminals, favouring neurogenic inflammation (15). These afferents respond to various mast cell products and express immune/inflammatory peptides and their receptors (20). Catecholamines, acting through alpha- and beta-adrenergic

receptors, mediate stress-induced increases in peripheral and central inflammatory cytokines and activation of the inflammatory nuclear factor-kB (NF-kB) signaling pathway (21). Classically, the SNS has a pro-inflammatory role (22) and is also involved in stress-induced remodeling of lymph node innervation favouring infection (23) thus revealing a link between behavioural factors, immune response and infection.

2) *Vagal inhibition*: The VN, classically has an anti-inflammatory role. Indeed, pro-inflammatory cytokines (IL-1beta, IL-6, TNF) released from the intestinal mucosa activate VN afferents which terminate in the NTS then relaying visceral information to activate the HPA axis (24). More recently, an anti-inflammatory role of VN efferents through the cholinergic anti-inflammatory pathway has been reported (25). Acetylcholine (ACh), released at the distal end of VN efferents, decreases the production of proinflammatory cytokines such as TNF by human macrophages through alpha7 nicotinic ACh receptor (a7nAChR) expressed by macrophages (26) (Figure 3). VN stimulation (VNS) attenuates the systemic inflammatory response to endotoxin (25) and intestinal inflammation (27,28). The VN also indirectly modulates immune activity of the spleen through its connections with the splenic sympathetic nerve (29). Smoking (e.g. nicotine) is protective in ulcerative colitis (UC) while deleterious in Crohn's disease (CD). This dichotomous effect results from the up- and down-regulation of anti-inflammatory $\alpha 7$ nAChR on colonic CD4 T cells induced by cytokines characteristic of the inflammatory milieu (30).

Stress decreases VN efferent outflow (16,31) and increases sympathetic outflow and adrenomedullary activity leading to increased norepinephrine and epinephrine levels (31) thereby inhibiting immune cell functions (32) and favoring intestinal inflammation.

3) *The prefrontal-amygdaloid complex and the immune system*: The activity level of the sympatho-vagal balance and the HPA axis, represented by peripheral measures such as heart

rate variability (HRV) and cortisol, are associated with the activity of the prefrontal cortex (PFC) and amygdala, respectively (33). The PFC contributes to negative feedback control of the HPA axis (34). The amygdala (i.e. central nucleus) is a target region for the control of the HPA axis and receives large inputs from the VN (35) that triggers the negative feedback on HPA axis. An inverse relationship between the plasma level of cortisol and the amplitude of HRV reflects the inhibitory influence of the PFC on amygdala (33). The hypoactivity of the PFC and the enhancement of amygdala activity are strongly influenced by stress (34). The PFC regulates peripheral immune cells through the autonomic and neuroendocrine pathways (36). The PFC controls the parasympathetic tone by modulating the VN efferent outflow. A dysregulation of this balance between the amygdala and the PFC induces an imbalance between the HPA axis and the ANS, as observed in IBD (37), and consequently a pro-inflammatory condition. Increased inflammatory markers [e.g., C reactive protein (CRP) and IL-6] are associated with decreased parasympathetic activity (decreased HRV), indicating that the cholinergic anti-inflammatory pathway counter-regulates inflammation (38).

4) Downregulation of the hypothalamic CRFergic system: An adaptation of the hypothalamic CRFergic system is observed in chronic stress (39). Chronic colitis suppresses CRF gene activation in the hypothalamus and plasma corticosterone level and dampens the counter-regulatory anti-inflammatory mechanisms during water-avoidance stress thus contributing to the stress-related worsening of colitis (40). A blunted HPA axis is associated with susceptibility to autoimmune/inflammatory diseases in Lewis rats in contrast to Fischer rats with an exaggerated HPA axis response (41). When Lewis and Fischer rats develop comparable TNBS-colitis, chronic stress more consistently worsens colitis in Lewis rats thus highlighting the protective role of endogenous brain CRF on the pro-inflammatory effect of chronic stress (42). In IBD a predisposition to a hyporeactive HPA axis and an inhibition of the central response to a chronic interoceptive stress may lead to colonic inflammation.

5) Role of the peripheral CRFergic system in inflammation

CRF ligands and receptors are also widely expressed in the GI tract (15,43,44) and may play an anti- or pro-inflammatory role. CRF receptors are present in different immune cells (e.g. macrophages, lymphocytes and mast cells) and CRF endogenous ligands locally secreted act as autocrine or paracrine modulators of inflammation. The CRFergic system expression is increased in experimental ileo-colitis (45). CRF2-deficient mice develop reduced intestinal inflammation to intraluminal *Clostridium difficile* toxin A (46) and peripheral CRF promotes inflammation of the terminal ileum (47). Both non-selective (alpha-helical CRF9-41) and selective (antalarmin) CRF1 antagonists have anti-inflammatory effects in *Clostridium difficile* toxin A-induced ileitis in mouse (48) favouring a role of CRF1 receptor, at least in part, in this effect.

Stress and CRF increase colonic permeability in rats (49) following activation of mast cells through a CRF receptor-dependent mechanism (50) via mast cell dependent release of TNF- α and proteases (51). This effect is potentially pro-inflammatory because penetration of luminal bacterial antigens triggers the activation of the immune system in the lamina propria.

CRF1,2 agonists exert a biphasic effect on macrophages with a suppressive effect of TNF release at the early phase and TNF production at the later stage of the inflammatory response (52). CRF peptides induce TLR4 expression through CRF2 (53). In contrast to CRF, Ucn 1 which binds to the same receptors has anti-inflammatory effects in TNBS-induced colitis in mice (54). The opposite effect of CRF related-peptides and their receptors could be attributed to their different distribution pattern and to the opposite effect of CRF1,2 on intestinal angiogenesis (55). CRF1 promotes intestinal inflammation, endogenous and inflammatory angiogenesis, whereas CRF2 inhibits these activities (56). The peripheral CRFergic system forms an interacting and balanced system and the differences observed among studies depend

on the model of inflammation and the receptors activated and the ligands; an imbalance of this system could favour GI inflammation.

6) The microbiota-brain-gut axis: Increasing data suggest a role of the gut microbiota in IBD (2). There is a bidirectional communication between the nervous system and commensal, pathogenic and probiotic organisms. Stress-increased intestinal permeability allows bacteria to cross the epithelial barrier to activate mucosal immune response (57) and to translocate to secondary lymphoid organs (58) to stimulate the innate immune system. Exposure of mice to a social stressor affects the structure of the intestinal microbiota and increases the circulating level of cytokines; antibiotics abrogate the stressor-induced increases in circulating cytokines (59). Changes in the intestinal microbiota reduce resistance to infectious challenge with intestinal pathogens (60). These data provide evidence for the interplay between stress, the intestinal microbiota and the immune response.

The SNS, through the release of catecholamines (e.g. norepinephrine), stimulates growth of bacteria (61). Stress-mediated changes may shift the microbial colonization patterns on the mucosal surface and alter the susceptibility of the host to infection; these changes in host-microbe interactions may also influence neural activity in stress-responsive brain areas (61).

The intestinal microbiota may act as a mediator in the communication between the gut and the brain (i.e. the microbiota brain-gut axis). Mice treated orally with *campylobacter jejuni* showed vagally mediated activation in the NTS in the absence of intestinal inflammation (62). Commensal microbiota can affect the postnatal development of brain systems involved in the endocrine response to stress. Indeed, an exaggerated response of the HPA axis to stress was observed in germ free mice that was reversed by reconstitution of the microbiota (63). Prevention of intestinal barrier impairment by a probiotic attenuates HPA response to an acute psychological stress in rats (64). In addition, germ-free mice show reduced anxiety-like

behaviour in comparison to specific pathogen-free mice, a phenotype accompanied by changes in plasticity-related genes in the hippocampus and amygdala (65), two key structures in the adaptation to the stress response. The intestinal microbiota influences central (i.e. hippocampal) levels of brain-derived neurotrophic factor (BDNF), which regulates dendritic architecture and spines, and behaviour independently of the ANS, gastrointestinal-specific neurotransmitters, or inflammation (66).

7) Effect of early-life events on colitis: The HPA axis is programmed by early life events and neonatal inflammatory stimuli exert long-term changes in HPA activity and immune regulation in adult animals (67). The modification of the stress axis early in development could favor a maladaptive control of the brain over peripheral immunity. Mother–pup interactions are an important factor to maintain a blunted HPA axis response (68). Maternal separation (MS), a model of early life stress, induces life-long hyperactivity of the HPA axis response to stressors (69) and an abnormal central CRFergic system (70). MS predisposes adult rats to stress-induced visceral hypersensitivity, hyperdefecation, dysfunction of intestinal barrier, increased HPA axis response, and anxiety (71,72). MS animals have an increased colonic adherence and penetration of bacteria into the lamina propria, a greater translocation to the liver and spleen, an increase mucosal mast cell density and cytokine mRNA expression in the colon due to alterations of the intestinal epithelial barrier (72,73). Neonatal stress causes marked alterations in the fecal microbiota of MS animals (74). A significant increase in the mRNA levels of TLR3,4,5 is observed in the colonic mucosa of MS rats with implications for susceptibility to infection/inflammation (75). Intracolonic infusion of TNBS induces a significantly higher inflammatory reaction in MS animals (73).

Early life stress in rodents can change methylation patterns in the genomic DNA, causing permanent alterations in gene expression in the brain. Hypomethylation of a critical cAMP-response element in the CRF promoter, a region critical for CRF transcriptional activation,

favors increased transcriptional responses to stress in MS rats (76). Deficient maternal care in rats increases glucocorticoid receptor promoter methylation leading to decreased expression in the hippocampus, a recognized target for glucocorticoid feedback (77).

8) *Effect of depression on colitis*: Some have argued for a causal relationship between depression and the immunological activation and hypersecretion of pro-inflammatory cytokines (IL-1, IL-6 and TNF- α), increases in peripheral blood chemokines and cellular adhesion molecules, and stress-induced NF- κ B (78). There is a link between early-life stress and depression that may predispose to increased inflammation both under baseline conditions and following stress (79). MS mice develop a behavioral pattern reminiscent of depression and are more susceptible to inflammation; this vulnerability is reversed by tricyclic antidepressants (80). Experimental sustained depression in mice is followed by impaired parasympathetic function and increased susceptibility to an experimental colitis that was reduced by desmethylinipramine, by a vagally dependent enhanced parasympathetic function (81).

Human studies

Altered psychological functioning pre and post IBD diagnosis

Whether or not depression and anxiety actually impact on the inflammatory state in IBD and secondarily on disease expression, it is well known from community studies that a great deal of the functional impairment and disability associated with health conditions is related to the presence of anxiety or depression (82-84). Subjects with more depressive symptoms exhibit enhanced inflammation to a stressor compared with those with fewer depressive symptoms (85). Individuals with IBD have lower quality of life (QOL) than the general population, as well as diminished psychological functioning and well-being (86-89). This seems to be more related to disease activity than merely having the diagnosis of IBD. A number of studies have

reported that psychosocial outcomes tend to be poorer during periods of active rather than inactive disease (90-94). Regardless of disease type, individuals with increased disease activity were more likely to report increased anxiety and depression while individuals with decreased disease activity over time reported reduced psychological distress (89). Psychological measures in chronic disease are dynamic. For instance, Lix et al showed that in following a cohort of IBD patients over a 2 year period there was a significant decrease in perceived stress, health anxiety, and pain anxiety, and a significant increase in pain catastrophizing over time (5). Again, the main driver of these responses was disease activity. Another aspect to consider is whether all IBD behaves similarly. Even within the cohort of persons with CD there is variation in psychological outcomes by disease phenotype. Lix et al found that study participants with penetrating or stricturing disease reported significantly lower scores on the Inflammatory Bowel Disease Questionnaire and the SF-36 physical health component than individuals with inflammatory disease (5). Nonetheless, the majority of the recent literature consistently suggests that disease activity rather than disease subtype is the key factor driving psychological outcomes (7, 88, 91, 95-97).

While psychological attributes may be a sequel to active disease, they may also contribute to health perception establishing a complex cycle. A Canadian population based cohort of IBD subjects (the same cohort used in the Lix study, (5)) was compared across a number of psychological and QOL domains to a cohort of Canadians responding to a nationally-representative random sample survey, the Canadian Community Health Survey (CCHS) (98,99). Psychological factors had a greater contribution to health perception for the IBD than the non-IBD sample. However, those with inactive disease were quite similar to the non-IBD sample, and had modestly higher mastery levels.

Distress levels were significantly higher for those with IBD (both CD and UC) than without IBD. However, those with inactive disease did not report any more distress than the

community sample. Less than 10% of those with inactive disease saw themselves as being in poor health, similar to the non-IBD sample. Hence, getting a patient into remission may benefit their psychological well being as well as their physical complaints. It is not having the disease per se that relates to psychological difficulties, but rather that disease activity is pivotal. Of interest in this study was that IBD subjects reported modestly higher levels of mastery than community controls. The study subjects had lived with IBD for an average of four years; and so perhaps having a chronic disease possibly strengthened their sense of mastery as they ‘weathered’ the illness. The same group of investigators studying the same IBD cohort over time found that longitudinal tracking of mastery was associated with modest increases over time with levels highest for those with inactive disease (5). So while living with chronic disease may have negative impacts psychologically, there may be psychological variables that are strengthened.

Persons with chronic disease may have higher levels of distress, health anxiety, and perceived stress, and it has been shown that persons with IBD have higher lifetime rates of panic, generalized anxiety, obsessive-compulsive disorders and major depression compared to control populations (100). Are these diseases a sequel to having a chronic disease, a prelude to the chronic disease or a combination?

A large scale epidemiological study of health records among residents of southern England with UC and CD found that much higher rates of treatment for anxiety or depression were seen in the first year following diagnosis of IBD than among residents without IBD (101). In a sample of patients with IBD seen in a gastroenterology clinic it was found that disease severity and psychological symptoms contributed independently to impaired QOL (7). Depression and anxiety in particular are thought to exacerbate chronic health conditions through a number of mechanisms, including decreased adherence to treatment recommendations, suppressed immune system functioning, and altered ANS (i.e. reduced

vagal tone with excessive sympathetic activity) or HPA activity (i.e. hyperactivity in ~ 50%) (100,102,103).

These psychiatric diagnoses may also be brewing prior to initial IBD diagnosis. Compared to community controls the 12 month prevalence of major depression in a population based cohort of IBD subjects was 9.1% vs. 5.5% (odds ratio, OR 1.72, 95% confidence interval, CI 1.07-2.76) (100). While IBD symptoms are emerging and diagnostic uncertainty prevails within 12 months from diagnosis, it is not so surprising that higher rates of depression are evident prior to diagnosis. Further, comparing IBD respondents with and without lifetime anxiety or mood disorder, those with a disorder reported lower QOL and earlier onset of IBD symptoms (29.1 years vs 33.1 yrs, $p=0.012$) and there was a trend toward earlier IBD diagnosis (101). Depression can negatively impact on the course of disease. For instance, one prospective study of IBD patients enrolled after a flare found that those with clinically significant depressive symptoms at baseline had relapses that occurred sooner (median time to first relapse was 97 days vs 362 days in those without depression, $p<0.05$) and more frequently during the following 18 months (104). In a prospective study of CD patients, major depression was a risk factor for failure to achieve remission with infliximab treatment and an earlier need for reinitiation of treatment (105). Alternatively, two studies found that higher levels of depression and anxiety during an active disease phase dropped with improvement in IBD (95, 106). Further, better psychological adjustment was associated with greater bowel and systemic health, increased activities engagement and symptom tolerance, less pain, less perceived stress, and fewer gastroenterologist visits in a clinic sample of persons with IBD (107). Pellissier et al. (108) have separated IBD patients according to their affective adjustment (a global psychological factor including anxiety trait and state, depressive symptomatology, negative mood, and perceived stress) and found a state of vulnerability in half of the patients, even in remission. In CD and UC patients, a negative affect was

associated with emotion-focused coping (less beneficial for good health), while only patients with a positive affect had similar coping strategies with controls, i.e. often more problem-focused coping. Consequently, the psycho-physiological vulnerability of IBD patients should be identified to consider the psychological needs in the follow-up of IBD patients in the remission period.

Is it possible that having a mood disorder would predispose to development of IBD? One study of 40 subjects suggested a higher than expected prevalence of depression among subjects with IBD (109). Walker et al not only compared 12 month prevalence of psychiatric disorders but also the lifetime prevalence in a population based cohort of 351 subjects with IBD compared to a population based study drawn from the same community (the CCHS) (100, 110). Compared to the community controls the lifetime prevalence of major depression in the IBD subjects was 27.2% vs. 12.3% (OR 2.20, 95% CI 1.64-2.95). About half of those with a mood disorder (54%) experienced a first episode of depression more than two years before the onset of IBD. Patients with major depression have increased concentrations of the HPA axis hormones, ACTH and cortisol, as well as increases in the cerebrospinal fluid measures of CRF (111,112). Depression induces an autonomic imbalance with impaired parasympathetic function and a dominant sympathetic drive (113). The impact these responses might have chronically in driving an intestinal immune response in genetically predisposed individuals is unknown but may provide mechanisms by which IBD follows sometime after the onset of depression.

A systematic review suggests a beneficial effect of antidepressants in IBD (114,115). Antidepressants for concomitant mood disorders in IBD seem to reduce relapse rates, use of corticosteroids, and endoscopies in the year after their introduction (116). Consequently, there is a need for prospective controlled trials to evaluate the effects of anti-depressants on disease course in IBD patients.

Stress and IBD

There is now compelling evidence that early childhood trauma and losses constitute major risk factors for the subsequent development of depression. Thus, the combination of genetics, early life stress, and ongoing stress may eventually determine individual susceptibility to psychiatric disorders, including depression (82,117). Basic research revealing an impact of stress in the response of the GI tract is not limited to animal studies. One fascinating study suggested that moderate stressors could alter human salivary mucosal secretory glands, which in turn affected microbial colonization (118). Hence stress could be linked to altered microbial flora interaction with the intestinal immune system.

There is consistent evidence that psychological factors play a role both in the pathophysiology and course of IBD and in how patients deal with IBD (119,120). One prospective study in a population based cohort of persons with IBD (n=552) evaluated whether having a stressful event and also the perception of stress as well as other factors (NSAIDs, antibiotics, infections) thought to be contributors to triggering flares of IBD were in fact associated with symptomatic flares (121). Subjects completed surveys on health issues every 3 months for one year. In any 3-month period, approximately 50% experienced some type of stress and the majority of reported stresses were everyday life stresses; family stress was the most commonly reported, followed by work or school and financial stress. Subjects were grouped by disease activity over time. Significantly more people in the persistently inactive disease group indicated they'd experienced no stressful events compared to those in the persistently active disease group. In terms of the association between variables experienced in one 3 month period and a symptomatic flare in the next 3 month period, only the psychological factors, including occurrence of a major life event, high perceived stress, and high negative mood during a previous 3 month period were significantly associated with the subsequent occurrence of a flare (121). This study complements the growing evidence from experimental

as well as clinical studies that stress exposure, including stressful events and perceived stress (the individual's view of their own level of demand relative to their resources), may contribute to relapse risk in IBD (93,94,122-124). In fact, on multivariate logistic regression analyses of these variables only high perceived stress (adjusted OR 2.40 95% CI 1.35, 4.26) was associated with increased risk of flare. This speaks to the bidirectional relationship between stress and symptomatic disease. Being symptomatic may exacerbate or even incite stress while being stressed may trigger symptomatic disease.

What is it about stress that could trigger a symptomatic flare? Activation of a stress response is highly dependent on the appraisal of the circumstance as stressful, which is influenced by individual difference factors such as coping strategies, life experience, and personal resources (121). A meta-analysis assessing the impact of stress on disease course in multiple sclerosis, also found that stress was associated with disease exacerbation (125). The key factor is the perception of stress, which incorporates the individual's appraisal of the demands created by stress in general and resources to cope with stress. Chronic stress, including caregiving and marital discord, and perceived stress are associated with increases in CRP and other inflammatory mediators (126). Perhaps acute stress may inhibit the HPA which would increase glucocorticoid resistance and reduce the endogenous immune response which can lead to a flare (127). Among 103 healthy women with high chronic interpersonal stress at baseline, their leukocytes displayed greater increases in mRNA for the proinflammatory transcription factor NF- κ B over the next 6 months. Elsewhere, chronic interpersonal stress at baseline was associated with increasingly pronounced IL-6 responses to lipopolysaccharide (LPS) (128). In a study of rectal mucosa of persons with UC compared to healthy controls stress increased LPS-stimulated cytokines, leukocyte and NK cell counts, platelet activation and reactive oxygen metabolites production and reduced rectal mucosal blood flow (129). Others have shown that sympathetic nerve fibers and sympathetic neurotransmitters are lost in

inflamed areas of the colon in both CD but not in non-inflamed ileum (130). So alternatively, stress may generate symptoms from the uninflamed areas where the sympathetic nerve fibers are intact. Perhaps, then, stress may contribute to spreading of the inflammatory lesion.

Recent reviews have concluded that stress has an impact on the course of disease but the jury is still out as to whether cognitive therapies or psychotropic medications can positively influence the course of IBD (131,132).

Exploration of the brain-gut axis in IBD

Neural response and brain imaging studies in IBD: Altered sensory processing has been described in IBD patients but in an opposite way to that evident in IBS. Whereas IBS patients were hyperalgesic to phasic rectal balloon distension, CD patients were hypoalgesic (133). Rectal perception was also attenuated in UC patients in another study (134). Thus the neural processing of painful stimuli may be distinct among IBS and IBD, including at a cortical level. Loci of activation and deactivation to visceral sensations of stool and pain have been studied in controls and in patients with IBD and IBS (135). Both activation and deactivation of particular regions of interest (e.g. anterior cingulate cortex, somatosensory cortex, frontal cortex) differentiated the three groups, as have profiles of patterned response across six of the regions of interest for control and IBD groups. Mayer et al. (136) have shown that chronic colonic inflammation was not necessarily associated with increased visceral afferent input to the brain during rectal distension. They observed a greater activation of limbic/paralimbic circuits in IBS and an inhibition of these circuits in quiescent UC and controls by the right lateral frontal cortex. More recently, Agostini et al. (137) observed a significant reduced blood oxygen level-dependent signal in quiescent UC patients in the amygdala, thalamic regions, and cerebellar areas in response to positive emotional stimuli thus arguing for disturbances of emotional circuitry in such patients.

Heart rate variability (HRV) is a non-invasive ECG-based technique which is now commonly used in GI physiology to assess autonomic imbalances (108,138) although very few data are available in IBD (108). Two principal components are identified: a high frequency (HF), which reflects phasic vagal activity, and a low frequency (LF) related to sympathetic and vagal outflows. The LF/HF ratio is used as an index of sympatho-vagal interaction. Both a diminished vagal and an increased sympathetic modulation of the sinus node may be reflected by a reduction in HRV. Several brain areas including the amygdala and the medial PFC that are involved in perceptions of threat and safety are also associated with HRV (139). Brain structures associated with immune modulation overlap those associated with cardiovagal modulation (140). High HRV is associated with greater PFC inhibitory tone and pharmacological inactivation of the PFC is associated with a decrease in vagally mediated HRV. Decreased vagal function and HRV are associated with increased overnight urinary cortisol, and increased pro-inflammatory cytokines and acute-phase proteins. The PFC and the amygdala are important CNS structures linked to the regulation of these allostatic systems via the VN (33). The parasympathetic nervous system tone as inferred from HRV is inversely correlated with inflammatory markers (e.g. CRP, IL-6) in healthy individuals as well as in those with cardiovascular diseases or major depression (141). Further, there is a gender difference in neuroimmunomodulation since the inverse association between HRV and CRP is 4.4 times greater in females than in males (142). In IBD, the sympatho-vagal balance varies according to the disease (CD vs UC) and HRV parameters must be interpreted in relation to negative or positive affect (108). In CD with a positive affect, an adapted high sympathetic activity was observed. In UC, a parasympathetic blunting was observed in the presence of a negative affect and an equilibrated sympatho-vagal balance in the presence of a positive affect (108).

Exploration of the HPA axis: Very few data are available on the HPA axis reactivity in IBD. Hyporeactive HPA axis function in response to a psychosocial stressor has been reported in IBD patients at diagnosis (143). An uncoupling between the HPA axis and the SNS has been demonstrated in IBD patients where serum cortisol did not correlate with plasma NPY, a marker of the SNS (37). Such an uncoupling has also been observed in patients with systemic lupus erythematosus, and rheumatoid arthritis (144).

Translational implications with therapeutic applications through the brain-gut axis

Anti-TNF therapy is a gold standard in the treatment of moderate to severe IBD (145). Another alternative therapy to conventional anti-TNF treatment, based on brain-gut interactions, would be the stimulation of the cholinergic anti-inflammatory pathway either pharmacologically or through VNS or nutrition (Figure 4).

Pharmacological stimulation of the cholinergic anti-inflammatory pathway

Galantamine: is a cholinergic drug used for the treatment of Alzheimer's disease, and a centrally acting acetylcholinesterase inhibitor and a positive allosteric modulator of nicotinic receptors, including $\alpha 7$ nAChR, which stimulates efferent VN activity, suppresses serum TNF and IL-6 levels and improves survival during lethal endotoxemia in mice (146). Thus galantamine could be used to suppress abnormal inflammation.

CNI-1493: is a tetravalent guanylylhydrazone which inhibits production of pro-inflammatory cytokines in macrophages through the inhibition of the phosphorylation of p38 MAPK, which plays a role in the translation of mRNA of pro-inflammatory cytokines such as TNF (147). Intracerebral administration of CNI-1493 significantly inhibited serum TNF levels during endotoxemia through the VN (148). CD patients treated with CNI-1493 (8 or 25 mg/m²) for 12 days showed a clinical response in 67% at 4 weeks and 58% at 8 weeks and a clinical

remission in 25% at week 4 and 42% at week 8. Endoscopic improvement occurred in all but 1 patient (149).

Alpha7 nAChR agonists: A double-blind placebo-controlled trial of the $\alpha 7$ nAChR agonist, GTS-21, has been performed in experimental human endotoxemia with assessment of the innate immune response. Volunteers were treated with either GTS-21 (150 mg t.i.d. for 3 days) or placebo and then administered a low dose (2 ng/kg) of LPS intravenously. Higher GTS-21 plasma concentrations were associated with significantly lower plasma concentrations of TNF- α , IL-6, and IL-1ra (150). GTS-21 pretreatment also strongly decreased the severity of an experimental pancreatitis in mice (151). Another $\alpha 7$ nAChR agonist, AR-R17779, has been used with success in a mouse model of postoperative ileus (152).

Vagus nerve stimulation

VNS is used in humans in the treatment of seizure disorders and depression (153). We and others have assessed VNS in inflammatory conditions focusing on the GI tract (27,28). In most of the studies VNS was performed in acute conditions and in vagotomized animals with VNS of the distal end of the VN. In contrast, we have shown that VNS chronically performed 3 h per day for five consecutive days in freely moving rats with TNBS-colitis improved colitis (28). These data have potential therapeutic applications for IBD patients (154), and a pilot study on VNS in CD is underway (Bonaz et al, unpublished data).

Nutritional reinforcement of the cholinergic anti-inflammatory pathway

High-fat enteral nutrition, through the release of CCK, stimulates CCK receptors on vagal afferents, and attenuates the inflammatory response in a model of hemorrhagic shock-induced TNF and IL-6 release. This effect was abrogated by vagotomy and CCK and nicotinic receptor antagonists which also suppressed the protective effect of high-fat enteral nutrition on inflammation-induced intestinal permeability (155). Consequently, high-fat enteral nutrition has potential therapeutic implication in IBD where TNF α and intestinal barrier dysfunction are prominent.

While complementary and alternative medicines are widely used in IBD (156), there is limited evidence of the impact of these approaches for the reduction of inflammation. Compassion meditation decreases inflammatory (IL-6) responses to a psychosocial laboratory stressor (157). Short-term meditation increases HRV and decreases skin conductance, both markers of increased parasympathetic tone (158). Tai Chi decreases SNS activity (159). Yoga techniques increase parasympathetic drive (160). A positive correlation between hypnotic susceptibility and autonomic responsiveness during hypnosis is observed with highly hypnotizable subjects showing a greater increase of vagal efferent activity than persons not susceptible to hypnosis (161). Few data are available on the use of hypnosis in IBD which may be a promising adjunctive treatment for IBD (162). There are no data on the effect of repetitive transcranial magnetic stimulation of the PFC in IBD; such an approach, by improving the cholinergic anti-inflammatory pathway, would be of interest. Targeting the peripheral CRFergic system would also be of interest in the management of IBD as an alternative and/or complementary treatment to the conventional therapeutic approach in IBD.

Conclusion

Increasingly knowledge gained from animal models exploring the brain-gut axis has provided potential insight into the management of human IBD. Depression and stress may both result from active IBD but may also play a role in triggering or magnifying symptoms in patients with IBD. The important symptoms of pain and fatigue, frequently reported by patients with IBD (163) are impacted by a patient's mental health. Completely abrogating the inflammatory state may not eliminate these symptoms (164,165). The placebo response in IBD is further evidence that IBD can be modulated by the patient's perception of events external to their intrinsic disease (166-168). While a number of factors may contribute to the placebo response in IBD treatment trials, the potential of the subjects' own expectations and response to the practitioner underscores the importance of cognition and patient experience in effecting clinical responses. In the past 15 years there has been a successful emergence of treatment strategies impacting on immune mediators such as TNF- α and α 4 related integrins that direct lymphocyte trafficking. The next decade will see the emergence of other therapies that will modulate other effector mechanisms of immunoinflammation. Therapies that modulate neural control of inflammation and mental health (which may impact on both psychoneural inflammation as well as well symptom perception) should not be overlooked. While these newer therapies are developed and studied there remains a need to study older available and often cheaper therapies. Rigorous studies of antidepressant pharmacotherapies as well as behavioural therapies are warranted. Since not all instances of active symptoms are accompanied by objective measures of inflammation in IBD, an assessment of both the anti-inflammatory effects as well as the symptom reduction effects is warranted. There are groups around the world that have incorporated biopsychosocial approaches to IBD management already (108,169). We anticipate that these approaches will become standards of care as

emerging evidence solidifies the importance of the brain-gut axis in orchestrating the inflammation and symptoms of IBD.

ACCEPTED MANUSCRIPT

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Figure Legend

Figure 1

Neural pathway of immunomodulation. ACh, acetylcholine; ACTH, adrenocorticotrophic hormone; AP, area postrema (a circumventricular organ); CRF, corticotropin-releasing factor, the principal neuromediator of stress mainly located in the paraventricular nucleus (PVN) of the hypothalamus; DMN, dorsal motor nucleus of the vagus (at the origin of the vagus nerve efferents: red arrow); EN, epinephrine; GC, glucocorticoids; HPA, hypothalamic (CRF)-pituitary (ACTH)-adrenal axis; LC, locus coeruleus (the principal brain noradrenergic nucleus located in the pons involved in stress); LPS, lipopolysaccharides (endotoxins); NE, norepinephrine; NTS, nucleus tractus solitarius (receiving vagal afferents: black arrow); PVN, paraventricular nucleus of the hypothalamus; RVM, rostral ventrolateral medulla; SNS, sympathetic nervous system. (From Pavlov VA, Wang H, Czura CJ, et al. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med* 2003;9:125–134).

Figure 2

Brain-Gut Interactions in inflammatory bowel diseases (IBD): the different actors and pathways through which stress may play a deleterious role in IBD. CRFergic, corticotropin-releasing factor, the principal neuromediator of stress, and CRF-related peptides (Urocortins) and their receptors (CRF1,2); HRV, heart rate variability, a marker of the sympatho-vagal balance i.e. of the autonomic nervous system; SN, sympathetic nerves; VN, vagus nerve.

Figure 3

The anti-inflammatory effect of the vagus nerve. A) Acetylcholine (ACh), released at the distal end of VN efferents, decreases the production of proinflammatory cytokines such as TNF by macrophages through alpha7 nicotinic ACh receptor (α7nAChR) expressed by

macrophages and other cytokine producing cells. Cytokine synthesis and release are prevented by inhibiting NF- κ B and by stimulating the JAK-STAT anti-inflammatory pathway. B) The VN also indirectly modulates the immune activity of the spleen through its connections with the splenic sympathetic nerve in the celiac ganglion to release norepinephrine (NE) in the spleen to activate the release of Ach by lymphocytes to inhibit, through $\alpha 7$ nAChR, TNF secretion by macrophages. Ach, acetylcholine; $\alpha 7$ nAChR, $\alpha 7$ nicotinic ACh receptor; JAK-STAT, janus kinase-signal transducer and activator of transcription pathway; LPS; lipopolysaccharides; NF- κ B, nuclear factor kappa B; TLR 4; Toll like receptor 4; TNF, tumor necrosis factor; TNF-R, TNF receptor; VN, vagus nerve.

Figure 4

Activation of the cholinergic anti-inflammatory pathway as an anti-TNF therapy through a pharmacological or nutritional approach as well as with vagus nerve stimulation (VNS) or complementary medicines. Ach, acetylcholine; $\alpha 7$ nAChR, $\alpha 7$ nicotinic ACh receptor; CCK, cholecystokinin; TNF, tumor necrosis factor; VN, vagus nerve.

Figure 1

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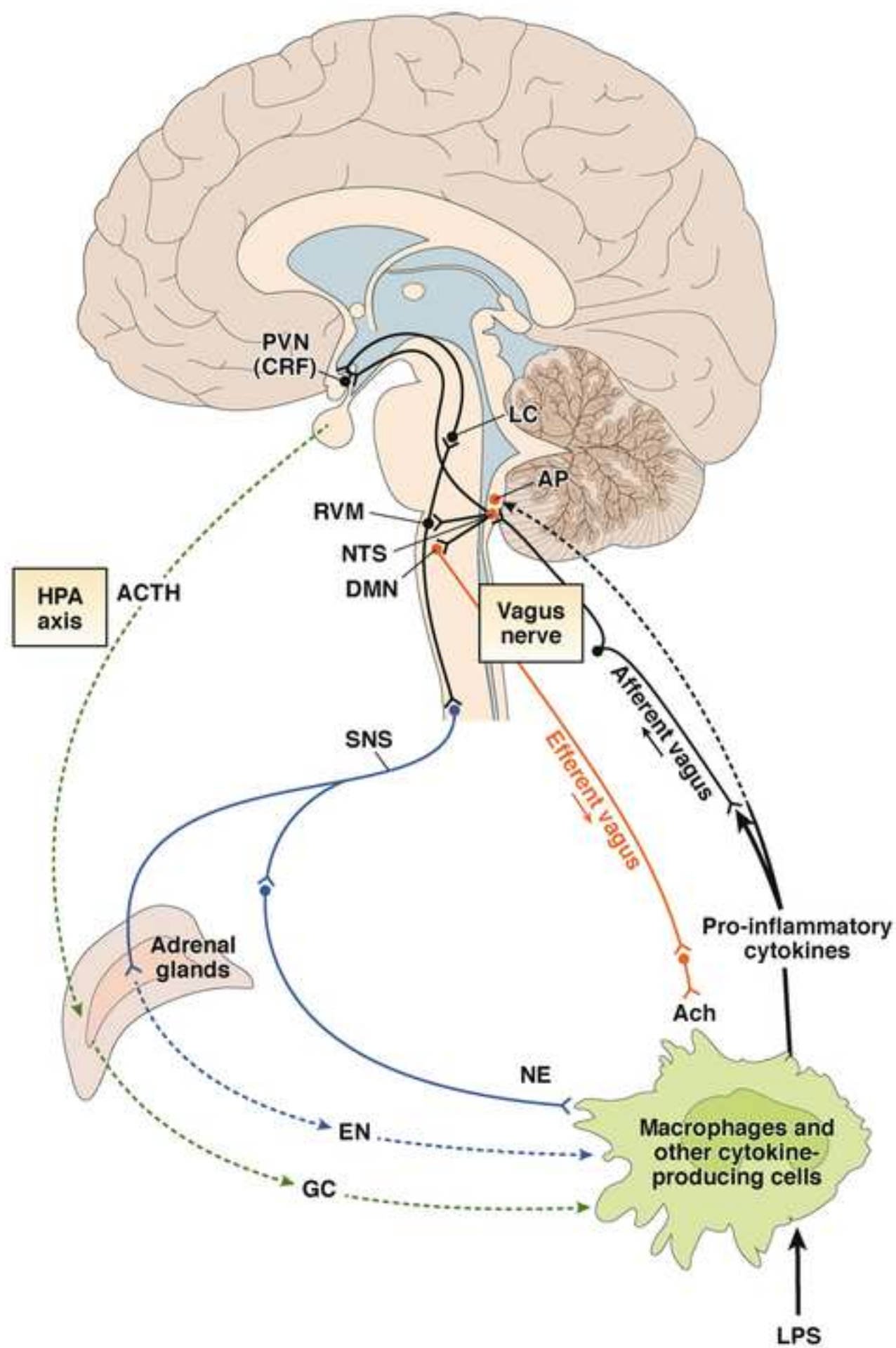
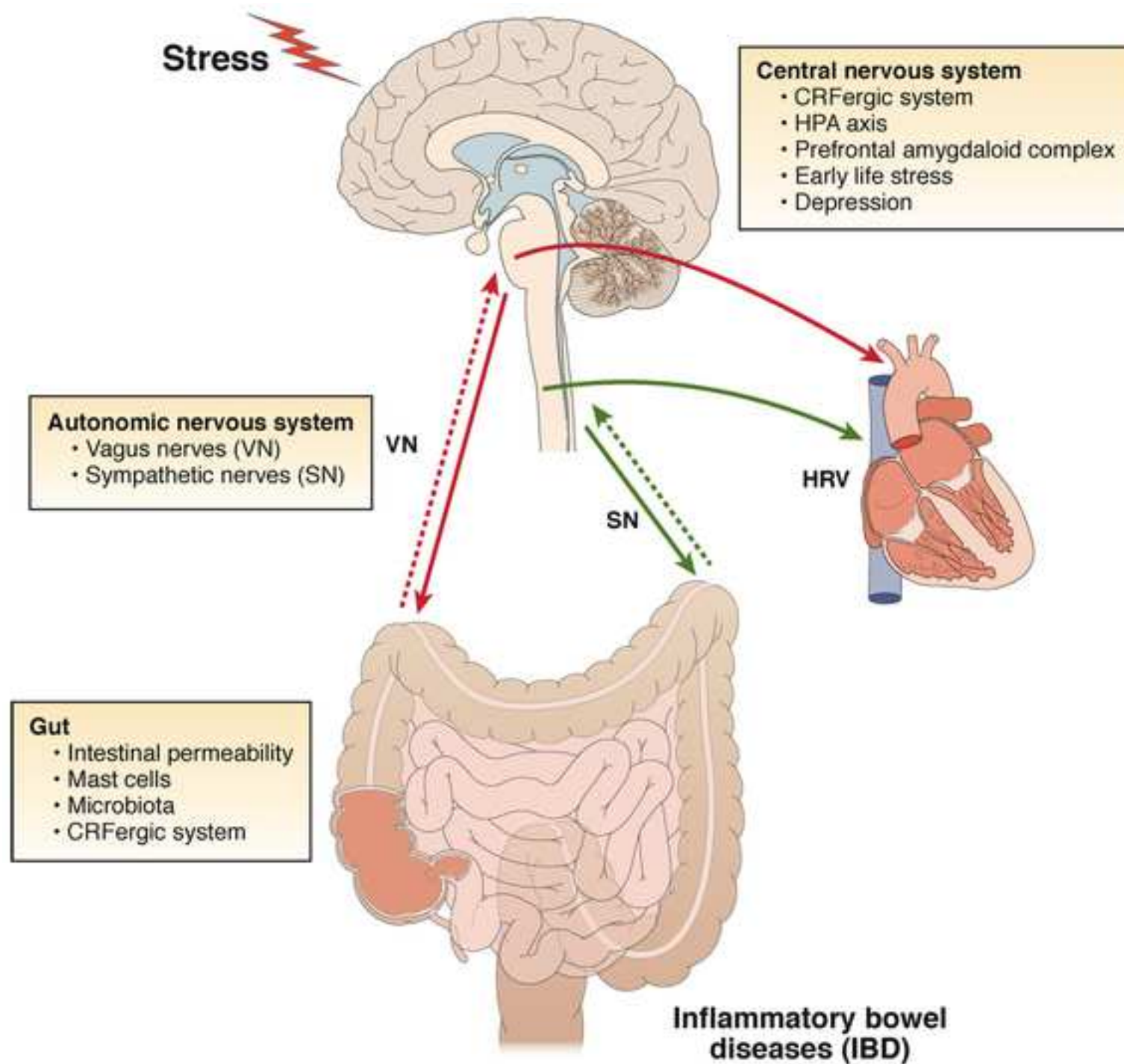


Figure 2

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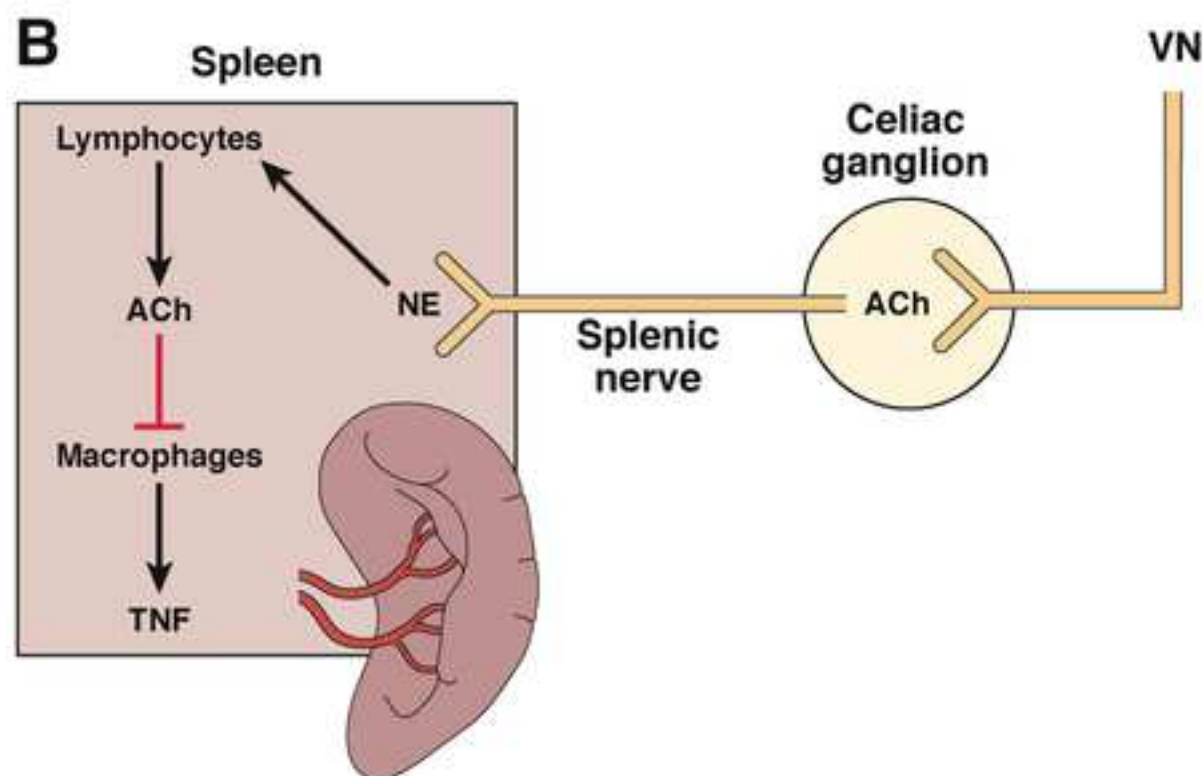
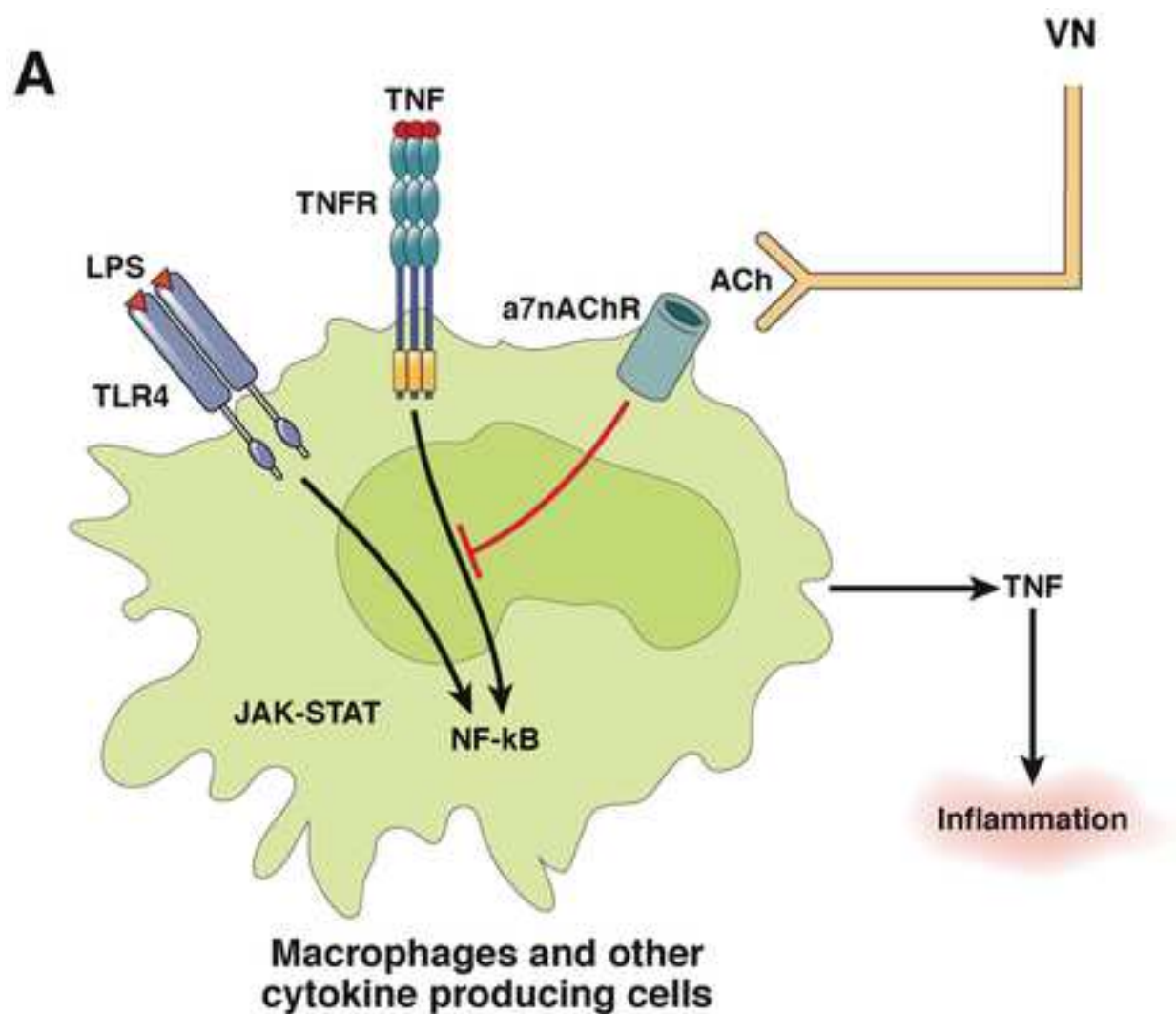


Figure 4

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