The microbiome-gut-brain axis in coeliac disease

Mechanisms for depression and anxiety co-morbidity

Master Thesis

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The microbiome-gut-brain-axis in coeliac disease: mechanisms for depression and anxiety co-morbidity

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CONTENT

ABSTRACT

Coeliac disease (CD) is an abundant autoimmune disease of the gastrointestinal (GI) tract. The pathogenesis of CD is triggered by administration of gluten, a common protein group in daily food. The resulting inflammatory reaction leads to lesions and villous atrophy in the small intestine. Besides the gastrointestinal manifestation, increasing awareness has risen on depression and anxiety disorder co-morbidity. Accumulating evidence suggests an important role of the microbiome-gut-brain axis in the development of these psychiatric conditions. In this paper, the recent literature on CD and the microbiome-gut-brain axis is reviewed. Moreover, folate deficiency, microbiome alterations and inflammatory mediators are discussed as causes of depression and anxiety in CD. Lastly, potential therapies against the psychiatric co-morbidity in CD are identified.

1. INTRODUCTION

Coeliac disease (CD) is a chronic immune-related disorder of the small intestine triggered in genetically predisposed persons by exposure to gluten (1–4). The clinical manifestation of CD ranges from asymptomatic to severely symptomatic, representing the degree of intestinal damage (2). CD is frequently present in Caucasian people, measuring prevalence up to 1 in 100 persons (5,6). The underlying pathological mechanism is triggered by the passage of large gluten peptides over the epithelium into the lamina propria (2). In the lamina propria, gluten peptides are deamidated by tissue transglutaminase (hTTG) and processed by dendritic cells (DCs) (2). Subsequently, the peptides are displayed on MHC class II receptors, which are predominantly HLA-DQ2 and HLA-DQ8 heterodimers (2). This activates pathogenic CD4+ T_h1 T cells, which start the production of proinflammatory cytokines (3). These cytokines lead to small intestine impairment by causing an inflammatory reaction in the epithelium and lamina propria, crypt hyperplasia and villous atrophy (3). These impairments disrupt the integrity of the tracts barrier function and lead to a variety of intra- and extra gastrointestinal (GI) symptoms (3).

Hippocrates (400 B.C.) described the importance of the GI tract in homeostasis with “a bad digestion is the root of all evil” (7). Although this was written in a period long before the major developments in western medicine, current understanding of the physiology of the GI tract proves him right in many ways (7). The intestines have a profound effect on the entire body, including the brain (7). Over the past centuries, many prominent scientists have postulated theories about the relationship between mood and the GI tract (7,8). The discovery of the enteric nerve system (ENS) halfway the nineteenth century was pivotal in the field (7). Consisting of a complexity similar to the central nerve system (CNS), the ENS is often described as the “second brain” (9). The found connection between the brain and the GI tract via the ENS provided a rationale for the earlier observed regulation of stress and emotion on gut functioning (7,10,11). Later it was found that this communication was bidirectional, explaining inter alia the hedonic feelings accompanied by food intake (12).

Over the past three decades, the link between the gut and the brain has been a point of major attention in gastrointestinal disorders (7). CD has a high co-morbidity of anxiety and depression, debilitating the lives of many patients beyond primary symptoms as diarrhoea and abdominal pain (13–15). Various mechanisms have been proposed for this distortion of the gut-brain communication, including malnutrition, inflammatory reactions in the gut and microbiome changes (13). A comprehensive review of the possible mechanisms could facilitate the development of new treatment options greatly. Therefore, we aim to discuss the co-morbidity and possible biological mechanisms of anxiety and depression in CD in order to identify new treatment strategies.

This paper deals with the general epidemiology and pathophysiology of CD in the first section. Secondly, the principles of gut-brain interactions in health and disease are described. The third part focuses on the function of the gut-brain-microbiome axis in CD in relation to the high psychiatric co-morbidity. The last section postulates future directions in the therapy of mental disorders in CD.

2. COELIAC DISEASE

2.1 Epidemiology

Since the introduction of reliable serological screening tests, the diagnosis and estimate of the

prevalence of CD is greatly improved (2). The test is based on an initial screening of IgA antigliadin and anti-hTTG antibodies (2). Subsequently, upon a positive test result, the diagnosis is confirmed via a biopsy in order to prove coeliac lesions in the small intestine (2). This well-established method has led to reliable values of the prevalence (5). The disease is frequently found in white people, measuring a prevalence of 1 in 99 schoolchildren in Finland (6). Comparable rates were found in children in Italy (1:106) (16) and in the adult population in the UK (1:87) (5). Of interest, Hispanic people are less susceptible, and the disease is rare among Africans (2).

2.2 Triggers, genetics and environmental factors

Several diseases are triggered upon introduction of gluten-rich products like wheat, barley and rye (2,17). The spectrum of gluten-related disorders can be divided in allergic, autoimmune and immune-mediated and the most common disorders of these groups are respectively wheat allergy, CD and gluten sensitivity (18). Besides the trigger, wheat allergy has few overlap with CD (18). Gluten sensitivity is a complex immune with no characteristics of either allergic or autoimmune origin (18). The disease is difficult to distinguish of CD, but does not show anti-hTTG antibodies (18). Because of the differences in pathogenesis, we will focus on the autoimmune disease CD and will leave the two other common gluten-related disorders out of consideration.

Gluten are chemically complex storage proteins, consisting of the subtypes gliadin and glutelin (17). Because of its proline and glutamine-rich profile, gliadin proteins are difficult to proteolyse by digestive proteins (2). This leads to large immunogenic peptides, which are a substrate for the enzyme hTTG (2). hTTG negatively charges the molecule, thereby increasing the affinity of the peptide for MHC class II subtype receptor HLA-DQ2 and DQ8 on T cells (2). Binding of these peptides drives the immune response in CD (4).

The interaction between the negatively charged gliadin peptides and the HLA-DQ2 and DQ8 molecules is the keystone of CD (3). Therefore, it holds no surprise that the genes encoding for HLA-DQ are strongly involved in the development of the disease (4). Multiple subtypes of HLA-DQ are known and these subtypes vary among individuals (19). The subtypes differ in affinity for peptides, thereby introducing a variance in immune response in individuals (19). Multiple diseases are associated with HLA variation (19). The association between the expression of HLA-DQ2 and HLADQ8 and CD is large, being expressed in 53 percent of the patients (19). However, the other 47 percent are dependent on other factors (19). Genome-wide studies have linked additional genes to CD (19). These genes mainly code for proinflammatory cytokines, inter alia interleukin (IL)-6 and -21(19).

Different environmental factors are linked to CD, although the mechanisms remain elusive (2). Early exposure to drugs and bacterial and viral infections can trigger the disease onset (20,21). Moreover, the microbiome is altered in CD and potentially plays a role in the pathogenesis (21). It is however unknown whether this is a cause or a consequence of CD (21).

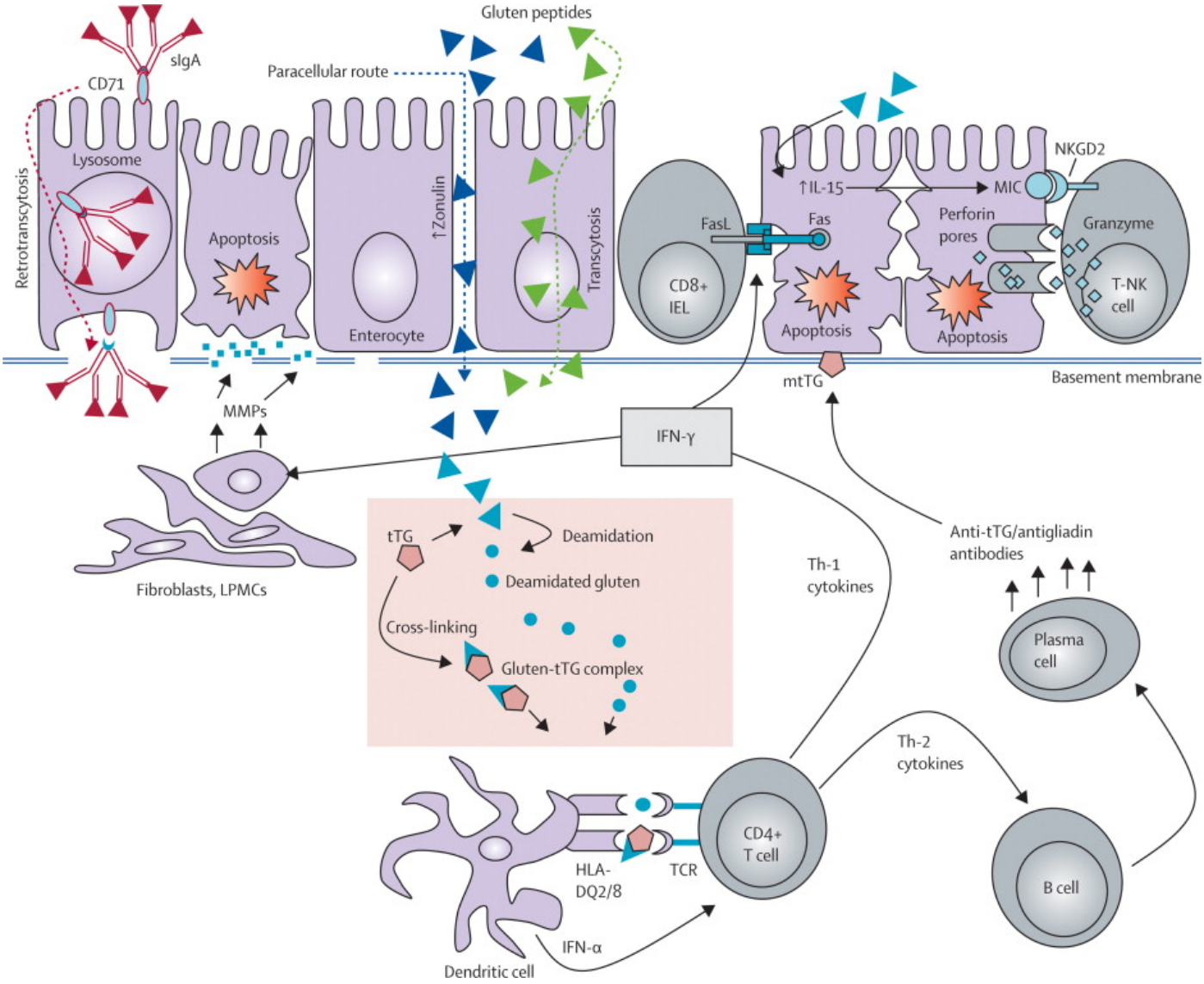

Figure 1. From di Sabatino and Corazzo, 2009 (2). The small intestine mucosal layer is damaged in CD patients as a result of several effector mechanisms. Gluten peptides are able to cross the epithelium transcellular and paracellular. In the lamina propria, hTTG processes gluten peptides, leading to the formation of several epitopes. The peptides are processed by DCs and presented on MHC class II subtype receptor HLA-DQ2 or HLA-DQ8. The presented gluten peptides activate T_h1 CD4+ T cells to secrete proinflammatory cytokines, including IFN- γ. These cytokines activate fibroblasts and mononuclear cells (LPMCs) to secrete metalloproteinases (MMPs), which are cytotoxic for enterocytes. Moreover, it stimulates CD8+ IELs and T-NK cells to induce apoptotic death of enterocytes. T_h2 CD4+ cells are activated and produce antigliadin and anti-hTTG (anti-tTG in text) antibodies. The antibodies are able to induce mucosal damage by destabilizing hTTG. See text for details.

2.3 Pathogenesis

Under physiological conditions, an individual does not develop an immune response against food allergens because of a mechanism named oral tolerance (22). Oral tolerance is induced by specialized mucosal DCs, which activate regulatory T cells (Tregs) upon exposure to food antigens (22). T-regs proliferate in the small intestine lamina propria and supress an inflammatory response by the secretion of Transforming Growth Factor β (TGF-β) (22). In CD, this mechanism is hampered on three essential points (fig. 1) (2). The first is the passage of gluten peptides over the epithelium (2). The second

crucial step is the modification and presentation of the processed gluten peptides (2,3,23). Third, the inflammatory effector mechanisms are not suppressed (3).

Gluten passage over the epithelium. In health and disease, incomplete digested gluten peptides are able to cross the epithelial barrier in different ways (17). The first manner is transcellular, which involves an interferon γ (IFN-γ) – dependent transocytosis mechanisms (2). Since IFN-γ levels are strongly upregulated in CD patients, the transport of gluten peptides is greatly enhanced, leading to an increased encounter of gluten peptide antigens with intestinal DCs (2). Transocytosis is also possible via an IgA driven mechanism (2).

Another, less well-established mechanism, is paracellular transport (3). An increasing body of evidence suggests the impairment of the barrier function in CD as a result of chronic inflammation, leading to an easier transport over the epithelium (24). For instance, it was found that expression of zonulin was significantly higher in CD patients (24). Zonulin is an enzyme that is involved in the opening of tight junctions, facilitating paracellular transport of gluten peptides (24). However, it is unclear to which extend this paracellular transport route delivers a substantial amount in the lamina propria (2). It is clear, however, that an increased exposure of DCs to gluten peptides facilitates the disease process (2).

hTTG modification and presentation. The ubiquitous, membrane-bound, calcium-dependent enzyme hTTG is involved in the modification of large gluten peptides (2). Besides the earlier mentioned charging of the peptide by deamidating glutamine, the enzyme also cross-links gluten peptides in immunogenic supramolecular complexes (4). Moreover, it incorporates histamine in the gluten peptides (2). The processing of these peptides leads to the formation of a wide range of epitopes, which can bind on specific T cell receptors (2). In CD, the expression of hTTG is upregulated, enhancing the ability to produce immunogenic epitopes (4).

Response mechanisms. CD4+ T cells are able to interact with the presented gluten epitopes, producing a T_h1-mediated response (2). Under physiological conditions, this production is inhibited by Tregs via inter alia TGF- β (25). In CD, CD4+ T cells are nonresponsive to the suppressive effects of Tregs and develop an immunological response (25). The nonresponsiveness of CD4+ T cells is caused by IL-15, which is produced by DCs (25). IL-15 is overexpressed in CD, but the mechanism behind this is currently unknown (25).

Prominent released cytokines are IFN-γ and –α, IL-6, -18 and -21 (3). Of note, the most abundant proinflammatory cytokine, tumour necrosis factor α (TNF-α) is not upregulated, as well as the most potent stimulator of IFN-γ production, IL-2 (2). This rather contradictory cytokine profile is caused by IFN-α producing activated by plasmoid DCs, which play a pivotal role in the differentiation of T_h1 CD4+ T cells and the production of IFN-γ (2). The release of these proinflammatory cytokines results in an increased secretion of metalloproteinases by fibroblast and lamina propria mononuclear cells, which exert a toxic effect on tissue matrix (2).

IL-15 is also involved in the expansion of CD8+ cytotoxic intraepithelial lymphocytes (IELs) (4). Moreover, CD8+ IELs and natural killer T cells are activated by IL-15 and IFN-γ to produce apoptotic factors like granzyme and perforin against enterocytes, leading to the characteristic villous flattening (2,4).

T_h2 CD4+ cells are also activated in CD and stimulate B cells to produce IgA antibodies against hTTG and gliadin (3). Anti-hTTG can cause more mucosal damage (3). Although this looks paradoxically, since hTTG is important in the formation and processing of immunogenic gluten epitopes, it is believed that the antibodies do not prevent this formation (3). However, the IgA antibodies do inhibit the formation of the active form of TGF-β into the active form (3). Therefore, besides CD4+ T cell unresponsiveness to TGF-β, the cytokine is also less activated in the case of IgA antibodies against hTTG (3). IgA can also destabilize the enterocyte cytoskeleton due to conformational changes in the membrane-bound hTTG and by stabilizing the enzyme in a catalytic advantageous confirmation (2). These processes lead to lesions and inflammation of the villi in the small intestine (2).

2.4 Clinical manifestations

In literature, several terms are available to characterize the clinical manifestations of CD. In this review, we use the terms as recommended by Ludvigsson et al. (1).

Asymptomatic CD. Patients with asymptomatic CD express antibodies against gliadin or hTTG, but fail to develop symptoms of the disease (1). Sometimes these patients show extragastrointestinal symptoms of anxiety and depression (1). When this is the case, the diagnosis should be redefined to subclinical CD (1).

Symptomatic CD. The severity of symptoms varies widely for CD patients (1,2). Some authors will therefore distinguish between minor and major CD (2), others between intragastroinstestinal symptoms and extragastrointestinal symptoms (4). Besides positive results on antibody screenings and biopsy, patients display various symptoms (1). These include dyspepsia, diarrhoea, malnutrition, cramps and peripheral oedema (1). Interestingly, anxiety and depression are also frequently found in patients with CD (13). The next section will cover this topic.

2.5 Co-morbidity of depression and anxiety

Typically, the research on CD has focused on the immunological background of gluten intolerance in an effort to find an effective therapy for the GI symptoms (13). However, due to increasing interest in the link between the gut and the brain, multiple epidemiological studies have been performed to investigate a possible relationship in CD with anxiety and depression (13). From the early eighties, case reports of CD patients with psychiatric disorders are appearing in literature (26,27). The most frequently occurring disorders are on depression, mood changes and anxiety (13,28).

Depression and mood disorders. The association of depression with CD is made by various investigators. According to a study performed by Hallert et al. in 1982 (26), patients with CD were significantly more depressed than healthy persons. This was later confirmed by an independent group in Italy (29), which also found a correlation with fatigue and depression in CD (30). However, small groups biased these investigations. The first population-based investigation that confirmed the earlier findings was performed by Ludvigsson et al. (13). They showed a significant increase in chance of depression in patients with CD, independent of confounding factors as age, sex, diabetes mellitus and thyroid disease (13). The latter two are interesting, because a study by Carat et al. suggested a possible link between thyroid disease and psychiatric co-morbidity in CD (31). Moreover, Garud et al. claimed a role for

diabetes mellitus in the psychiatric symptoms found in CD (32). According to Ludvigsson et al., however, these factors were not causative (13).

In the same study, Ludvigsson et al. failed to show a significant difference between healthy persons and CD patients in the occurrence of bipolar disease (13). Suicide risk is claimed to be increased in a subsequent study (33).

Interestingly, some investigators report that reduced quality of life and depressive symptoms are independent of the exposure to gluten, as measured in patients on a gluten-free diet (GFD) (34,35). However, later follow-up studies indicate the opposite, claiming complete remission of major depressive episodes after discontinuation of gluten rich diets (36–38). This was indirectly confirmed by a study with CD patients on GFD, who did not show more depressive symptoms than healthy persons (39). It is currently not clear whether GFD is able to reduce depressive symptoms. In favour of an on-going depression, Lee et al. demonstrated lesions and villous atrophy in patients with CD even after GFD (40). Given the chronic inflammation that appears in these patients, it is well possible that the immunological reaction influences brain, as will be described in the next section.

Anxiety Disorders. Besides depression, anxiety disorders are the most commonly found psychiatric disorders in CD patients (15,28,29). As described in some studies on depression, the introduction of GFD leads to a significant reduction of anxiety (35). Social phobia, a condition characterized by fear and/or avoidance of situations that involve possible scrutiny by others (41), is shown to be significantly higher in patients with CD in comparison to healthy persons. Moreover, a higher prevalence of panic disorder in CD is reported by Carta et al. (31).

3. MICROBIOME-GUT-BRAIN AXIS

The microbiome-gut-brain axis can be of great use in the understanding of psychiatric co-morbidity in GI diseases, including CD. In this section, the general mechanisms of microbiome-gut-brain signalling are explained.

3.1 Brain to gut communication

The brain mainly communicates with the GI tract via the autonomic nervous system (ANS), the hypothalamic-pituitary-adrenal (HPA)-axis and the sympatho-adrenal axis (7,10). The output originates from the amygdala and the hypothalamus, which on their part receive input from multiple cortical regions (7,42).

The descending projections of the ANS are divided in a sympathic and parasympathetic branch (7,43). The parasympathic output can be divided in vagal and sacral divisions, which stimulate motility and gastric acid secretion in respectively the foregut and hindgut (7). Moreover, it promotes the secretion of serotonin and histamine from the enterochromaffin cells (44). The cholinergic transmission of the parasympathic system has also been shown to modulate the immune response by binding to nicotinic receptors on macrophages, thereby downregulating the inflammatory response (45).

The sympathic innervation of the GI tract is inhibitory (7,42,43,46,47). Its neurons can be divided in three subclasses, including postganglionic vasoconstrictor neurons, motility inhibiting and secretion inhibiting neurons (43). The sympathic branch functions by reducing the cholinergic transmission at parasympathic neurons and contraction of sphincteric muscles (43,47). Moreover, accumulating evidence suggests a role in the immune response (7). In an experiment performed by Goal et

al., the investigators found that noradrenaline downregulates Toll-like receptors (TLRs) on intestinal epithelial cells infected by parasites (48). This was confirmed in a mice experiment, in which the animals were infected with a parasite and subsequently exposed to cold water in order to induce stress (48). The stress –induced innervation of the sympathicus led to the downregulation of proinflammatory cytokines and TLRs on intestinal epithelial cells (48).

The functioning of the gut can be seen as a hierarchy of reflexes (7,49,50). Gut function is partially regulated on a local level, involving enteric reflexes on chemical and mechanical stimuli (49,51). On the other hand, ascending interoceptive signals are also able to directly trigger ANS output reflexively (49,51). These reflexes can be overwritten by the cortical structures via the amygdala and hypothalamus in response to environmental factors, body homeostasis threats and severe emotions (7). The ability of overwriting locally triggered ANS functioning is the cause of changes in secretion, motility and immune activity in the GI tract in response to emotional distress (51,52). Prolonged shifts in ANS functioning can lead to chronic changes in peripheral target cells, including reduced receptor expression on the epithelium and immune cells (11). Recent investigations suggest this is the cause of tonic ANS dysfunction, which is frequently found in depressed patients (7). These patients report diarrhoea, obstipation and other problems in the GI tract (53).

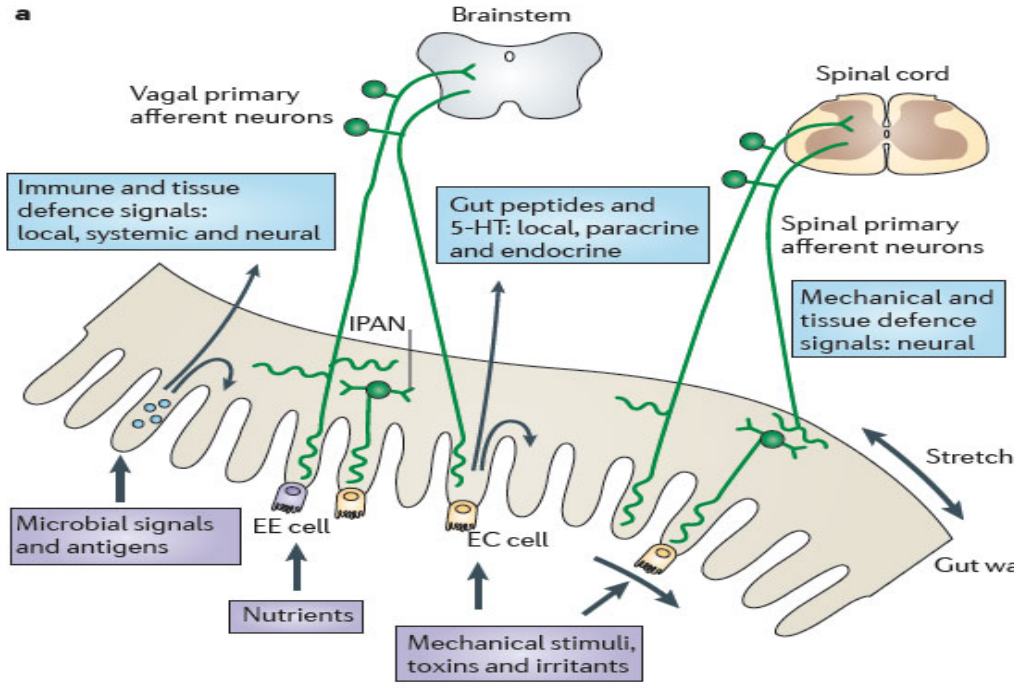

Figure 2. Adapted from Cryan and Dinan, 2012 (12). Gut to brain communication. The gut communicates with the brain via primary afferent neurons, para- and endocrine cells (EE- and EC cells) and immune cells. Various factors modulate these cells, including the microbiome, antigens, nutrients, mechanical stimuli and toxins. See text for details.

3.2 Gut to brain communication

The ENS is an incredible network of multiple classes of neurons, including between the 200 and 600 million cells, a number that equals the amount in the spinal cord (7). The high number of neurons is necessary to receive information of the largest part of the body surface, interfacing two-thirds of the body's immune cells and over 100 trillion commensal bacteria, which will be discussed later (12). Besides

this, the ENS also senses the hormones of thousands of endocrine cells (54). Given the wide range and diversity of signals, it is no surprise that ENS signalling has a profound function in homeostasis (50). The sensory system in the gut encompasses three mechanisms; signalling via primary afferent neurons, immune cells and enteroendocrine cells (fig. 2) (7).

Primary afferent neurons. The neurons of the ENS are classified into two groups according to the location of the cell body (7). Extrinsic primary afferent neurons (EPANs) are located in the spinal cord and nerves vagus (55). The EPANs are organised in modality-specific regions and deliver information from the gut to the brain (7). Intrinsic primary afferent neurons (IPANs) are present in higher numbers then EPANs and form extensive networks of self-reinforcing networks in the gut wall (7). Whereas the EPANs provide information to the CNS, leading to higher-level reflexes, the IPANs transduce local reflexes in response to different stimuli (7). IPANs can be mono- or polymodal (7).

Both groups express chemo- and mechano-sensitive receptors and ion channels, enabling the neurons to transduce several types of signals (56). EPANs have mechano-sensitive ion channels of different subtypes, which are able to signal contraction, distension and movement of the gut (56). Interestingly, under physiological circumstances not all mechano-sensitive ion channels are activated, but are sensitised during inflammatory reactions (57).

Endocrine and paracrine cells. The gut is the largest endocrine organ of the body and contains over 20 enteroendocrine cell types (54). The output of enteroendocrine cells is important in the regulation of the digestive system via the ENS and in mediating CNS processes by endocrine and paracrine signalling, which are received and conducted by afferent neurons of the nervus vagus (54). Enteroendocrine cells express multiple receptors on their surface, making them sensitive for various stimuli (7). The most prominent receptors are shearing force detecting mechano-sensitive ion channels (56), fatty acid sensitive G-protein coupled receptors (GPCRs) (54) and TLRs (48), which are able to detect microorganisms and their products. Neuroendocrine signalling in the gut is involved in the detection of satiety via cholecystokinin, glucagon-like peptide 1 (GLP1) and peptide YY, the feeling of hunger via ghrelin and nausea via serotonin.

One of the most prominent enteroendocrine cell types is the enterochromaffin cell (44). Containing serotonin as its main product, the cell is responsible for the storage of 95 percent of the body's serotonin (44). The enterochromaffin cell releases its content under movement and pressure, which is a result of mechano-sensitive ion channel activation (44). The released serotonin is known to be involved in the local activation of intestinal peristalsis and the secretion of gastric acid (7). Clearly, it is an attractive idea to suggest a role for enterochromaffin functioning in the influence of the gut on behaviour (58). However, at this moment, no study can confirm this (7).

Immune cells. The large surface of the GI tract opposes a unique challenge in the maintenance of immunity and tolerance. 70 – 80 Percent of the immune system is present in the gut associated lymphoid tissue, only one epithelium layer away from the enormous amount of microorganisms living in the intestines (12). Under physiological circumstances, the immune cells is hyporesponsive towards commensals (7,12). Differences between commensals and pathogens are detected with a wide range of pattern recognition

receptors, inter alia the earlier mentioned TLRs (54). The detection of antigens and microorganisms over the barrier, that the epithelium opposes, is enabled by specialized lymphoid structures in the lamina propria (12). These lymphoid structures, which include Peyer's patches, facilitate two kinds of transduction mechanisms. One way is the direct interaction of dendritic cells via their dendrites, which extend through the epithelial tight junctions (7). Moreover, specialized microfold cells are able to transport antigens and microorganisms via transocytosis towards DCs within the Peyer's patches, which could lead to an immunological reaction (7). In close proximity of the immune cells are vagal neuron terminals, which are able to locate a multitude of immune mediators, including histamine from mast cells and various cytokines (7). Vagal afferent neurons project via lamina I of the dorsal horn towards the parabrachial nucleus, which signals to the amygdala and hypothalamus, the behavioural region of the brain. Besides the influence on neuronal signalling, cytokines are also able to exert an effect the functioning of enteroendocrine cells, resulting in alterations in hormone and signalling molecule expression (59).

3.3 Involvement of the microbiome

The gut microflora consists of 10^{13} - 10^{14}, a number that is 10 times higher than the amount of cells present in the human body (12). In the past years, the awareness of the pivotal role of the microbiota in homeostasis is rapidly increasing (8,10,12,60,61). Investigators have shown the importance of gut bacteria in a proper development and functionality of the immune system (62), as well as in the degradation and absorption of nutrients, the distribution of fat, gut motility regulation and the general barrier function of the GI tract epithelium (63).

Current estimations of the amount of species in the gut vary greatly, but the general consensus is momentarily that it is inhabited by over 1000 species (64). The colonisation of the digestive tract is postnatal upon exposure at birth (12). In the early months, the bacterial composition of the gut is maternal (65). After 1 year, the microbiota have been developed into an adult-like composition (65).

The composition of bacterial communities can vary greatly among individuals (64). Studies have shown that genetics and feeding patterns are important factors in the development of the community (65). A stable and balanced composition is essential in homeostasis (12). The microbiota produce and secrete a large variety of different molecules, of which some are able to interact with immune, endocrine and neuronal cells. Given the importance in homeostasis, recent studies have focussed on the effect of the microbiota on behaviour (12). It is clear that the microbiome exerts an important effect via modulation of the earlier mentioned endocrine, immune and neuronal signalling pathways (7). By these pathways, the microbiome is able to communicate via the gut to the brain, making it an important player in the gut–brain axis (12). The brain is also able to influence the microflora composition, as is seen in a study by O'Mahony et al. (66). In this study, stress was induced in rhesus monkeys (6-9 months old) by maternal separation (66). This resulted in a significant decrease in faecal lactobacilli, indicating an effect of stress on microbiota composition (66). These findings have led to the concept of the microbiome–gut–brain axis (12,60,67). Although the stream of signals from afferent neurons, enteroendocrine cells and the immune system to the

brain is continuously, only a small portion is actively perceived (7). This is mostly information that requires a conscious response, for instance defecation (7). However, the GI tract information, that is processed subliminally, is also able to influence memory, behaviour and emotions (42). The most prominent examples are hedonic feelings after food intake and aversive reactions against food and drinks that earlier caused intoxication(7).

Disruptions of the GI tract can influence neuroendocrine, neuroimmune and neuronal signalling, which is likely to cause behaviour problems (42). This is perfectly illustrated by recent investigations on mental disorders in GI diseases (68,69). Irritable bowel syndrome (IBS), functional dyspepsia (FD) and CD are diseases of the GI tract that are associated with psychiatric disorders as depression and anxiety (69).

Several theories about the co-morbidity of anxiety and depression have been postulated (69). Some investigators believe the higher rate of depression and anxiety among CD patients is caused by a general reduction in quality of life found in chronically ill patients (14). However, giving the rapidly expanding knowledge of the interactions between the gut and the brain, it holds no surprise that several explanations have been sought in this network. In the following, we will discuss the evidence of some frequently postulated biological mechanisms behind depressive and anxious behaviour in CD.

4. MECHANISMS

4.1 Hyperhomocysteinemia and folate deficiency

The prolonged inflammation of the small intestine of CD patients frequently leads to villous atrophy (3). The impairments in the epithelial layer result in malabsorption of nutrients, which is the cause of various secondary symptoms in CD (3). Multiple studies in CD patients report low plasma levels of folate as a consequence of malabsorption (40,70–73). Folate, or vitamin B_9, is gained from dark leaf vegetables and fruits (74). After absorption, liver enzymes reduce folate in several compounds of which L5-methyltetrahydrofolate (L-5-MHTF) is the most biological active (71). L-5-MHTF is responsible for the conversion of homocysteine into methionine (71). A deficiency of folate therefore ultimately results in hyperhomocysteinemia (75). High homocysteine plasma levels are linked to various disease states, including depression, anxiety, heart failure and osteoporosis (75).

In 1967, Carney first described significantly lower folate levels in psychiatric patients with major depression (76). Later, various investigators have confirmed the link between low folate levels, hyperhomocysteinemia and psychiatric disorders (75,77,78). A study by Sachem et al. found a correlation between these plasma levels and psychiatric disorders in a middle-aged community (77). The same results were found by a study of Tiemeier et al. (74) In contrast, Bjelland et al. only reported an association between hyperhomocysteinemia and depression (78). The observational studies are indirectly confirmed by the finding of Kronenberg et al. that low folate levels correlate with low serotonin levels in the brain of mice, a common feature of depression (79).

Folate deficiency and hyperhomocysteinemia in CD have been reported since the beginning of this millennium (70). A study by Dickey et al. demonstrated a significantly lower plasma level of folate and higher homocysteine blood concentrations in untreated patients in comparison to CD patients on

a GFD and healthy people (72). This was in line with earlier results obtained by Hallert et al., who showed the same effect in both treated and untreated CD patients (70).

Taken the evidence on low folate levels, hyperhomocysteinemia and disorders together with the various studies reporting these plasma levels in CD patients, malabsorption of folate might be a plausible cause of psychiatric co-morbidity in CD.

4.2 Microbiome alterations

Given the influence of the microbiome on behaviour in healthy people, it is clear that alterations in the microflora can result in problems (12). Recent studies have investigated the effect on behavioural patterns (12). A study by Suds et al. in germ-free (GF) animals gave rise to the first indication on the effect of the microbiome (80). His group showed a marked increase in stress response in GF animals, which was reversible by introducing normal gut flora (80). Later studies in GF animals found a marked reduction in anxiety and depression (12,60,81). Several modes of action have been proposed to explain the observed phenomena (12). It is clear that immunological and neurological signalling is affected by microbiome alterations (12). Different bacterial enterotypes produce different metabolites, of which some are active signalling molecules (12). Short fatty acids, choline and bile acid are essential in host health, but concentrations can vary when the microbiome is altered (12). The same accounts for the neuroactive metabolites GABA, noradrenaline, dopamine and serotonin produced by the gut flora (12). These (neuro-)metabolites are active signalling molecules, which can be detected by EPANS, IPANS, enteroendocrine and immunological cells (50). The variation can thus have profound effects on the gut-brain signalling and eventually result in depressive and/or anxious behaviour (12).

The gut microbiota are an important player in the serotonin formation pathway (82). The precursor of serotonin, tryptophan, can be converted to either serotonin or kynurenine (82). Metabolism of tryptophan in kynurenine is mediated by indoleamine-2,3-deoxygenase (IDO) and occurs in the gut (12). Serotonin conversion occurs in the brain and is dependent on tryptophan plasma levels (12). When the tryptophan concentrations are low, brain concentrations of serotonin are decreased (12). Some investigators suggest that alterations in the gut flora are able to upregulate the expression of IDO, thereby facilitating the metabolism of tryptophan into in kynurenine (12). The increased activity of the kynurenine branch of tryptophan metabolism leads to a decrease in tryptophan plasma levels (82). A lowered tryptophan level is indeed found in patients with CD (27,83,84), although this is not yet directly linked to bacterial composition. It is, however, clear that reduced tryptophan plasma levels are associated with depression (82).

Microbiome alterations are commonly associated with GI diseases, especially with IBS (68,85). A changed microflora is also reported in CD, although this is mostly coupled to disease progress and not psychiatric co-morbidity (21,86–92). Variations in the microbiota have been described in faeces and duodenal biopsies using different techniques (21). Investigators have shown an increase in Bacteroides and Clostridium leptum groups in faecal samples, as well as E. Coli and Staphylococcus in CD (90). The Lactobacillus colony was found to be reduced in treated patients in comparison to healthy persons (91). This also accounts for the amount of Bifidobacterium and B. longum species (89).

The presented data suggest that a changed microbiome can play a role an explanation of the co-morbidity of psychiatric disorders in CD. However, more research is required to directly associate depression and anxiety with an altered microbiome in CD.

4.3 Mucosal inflammation and irritation

Besides causing malnutrition, prolonged expression of proinflammatory cytokines can induce mood changes and anxiety (93). Sukoff Rizzo et al. recently reported that IL-6 administration causes a depressive-like phenotype in mice, which can be improved by the administration of IL-6 antagonists (94). Other cytokines, including IFN- γ, have also been implicated in the development of depression and anxiety (95). Involved pathways are the downregulation of brain derived neurotropic factor (BDNF), the formation of free radicals and the induction of the HPA-axis by stimulating the release of corticotrophin releasing factor (CRF) (95). These mechanisms are all found in depressive patients and a large body of evidence underlines the importance of these processes in the pathogenesis (93). Moreover, these cytokines are known to stimulate IDO, the enzyme that converts tryptophan into kynurenine and other catabolites (95). This mechanism might be another explanation for the low tryptophan plasma levels found in CD patients, which are associated with depression (84).

5. TREATMENT OPTIONS

It is currently unclear to which extend GFD can alleviate depressive and anxious symptoms in CD (35,38,39). Although GI symptoms often wore off, data on depression and anxiety are contradictory (35,38,39). Since inflammation continues (2), microbiota composition remains altered (91) and folate levels stay low (40), even after GFD, this provides a rationale for treatment intervention. Besides the symptomatic alleviation of antidepressiva like selective serotonin reuptake inhibitors (SSRIs), a more mechanism-based approach can be chosen in order to alleviate the depressive and anxious symptoms.

5.1 Folate therapy

A logical response to low folate plasma levels is the introduction of folate (96). Orally, this can be formulated as L-methylfolate or folic acid (97,98). Fava et al. demonstrated symptom relief in depressive patients treated with the combination of antidepressants with L-methylfolate (98). This was in line with a later study in stress-induced depressive mice, in which depression was prevented via folic acid (99). Folate deficiency and hyperhomocysteinemia are also associated with atherothrombotic vascular diseases, and from this background a folate supplementation was given to CD patients (73). This increased folate plasma levels greatly, but unfortunately, the investigators did not measure depressive and anxious endpoints (73). A clinical trial in patients with CD and depression or anxiety is recommended.

5.2 Probiotics

Probiotics have proven to be beneficial for the host in many diseases and conditions, including IBS and psychiatric disorders (12). Recently, a clinical evaluation of a Lactobacillus Helveticus and B. Longum cocktail revealed anxiety-reducing properties and stress lowering effects in patients with phobia and stress (100). This same cocktail was found to be effective in decreasing depression in rats

who suffered from post-myocardial infarction, making it a good candidate in the treatment of CD (101). The mechanisms behind the effect of the Lactobacillus Helveticus and B. Longum cocktail are yet to be elucidated, but it is proposed that it functions via the dampening of the pro-inflammatory and oxidative effects in the gut, as well as increasing the nutritional state (12). This mechanism would fit perfectly in our current understanding of the mechanisms behind psychiatric co-morbidity in CD.

Another recent study reported an anxiety- and stress-reducing effect of Lactobacillus rhamnosus in mice (102). Moreover, it was demonstrated that the expression of $GABA_A$ and $GABA_B$ receptors in specific sites of the brain was altered by the lactobacilli (102). Of interest, this effect was not established after vagosectomy, indicating a nervus vagus dependent effect in the signalling of Lactobacillus rhamnosus (102).

More evidence of the effectiveness of probiotics in the improvement of depressive and anxious symptoms was given by data on B. infants (103). It was found that this probiotic cocktail in the rat and mice exerts anti-depressant-like effects by reducing the inflammatory reaction in the intestines and restoring the tryptophan levels in the blood (103).

Although some of these data clearly show an effect of probiotics in the treatment of depressive and anxious symptoms, it is difficult to compare the effect of one bacterial strain to the other. Moreover, efforts must be made to elucidate the mechanisms behind the effect of probiotics on behaviour in order to recommend probiotics in the treatment of CD.

5.3 Anti-inflammatory agents

Not specifically aimed at the psychiatric co-morbidity in CD, but rather on the on-going inflammatory reaction in the GI tract, anti-inflammatory agents can play a role in the reduction of symptoms in psychiatric disorders (2). Inhibiting the major effector cytokine IFN-γ can result in the remission of chronic inflammation (2). Anti-IL-6 antibodies are shown to be effective in vivo in depressed mice (94) and can be taken in consideration for further development. Moreover, treatment should focus on the silencing of gluten selective T-cells by CD3, CD4 or CD25 antibodies (2).

6. CONCLUDING REMARKS

Our knowledge of the effect of the microbiome-gut-brain axis in CD is rapidly expanding. This has let to the identification of several biological mechanisms that might play a role in the co-morbidity of anxiety and depression in CD. The co-morbidity of anxiety and depression is not restricted to CD, but accounts for several chronic diseases, including asthma, IBS and rheumatoid arthritis (RA) (104–107). Some investigators are therefore tempted to see it as a general feature of chronic disease and contribute psychiatric co-morbidity to difficulties of the patient to cope with their disease (14). Although we recognize the importance of psychological factors, there is a large body of evidence on biological factors.

Common denominators on the development of depression and anxiety co-morbidity are found between different chronic diseases (104). RA is a systemic inflammatory disease with high plasma levels of pro-inflammatory cytokines, including C-reactive protein, TNF-α and IL-6 (107,108). These cytokines are believed to be involved in the pathogenesis of depression and anxiety (95,107). IL-6 is upregulated in CD and asthma as well (2,106), suggesting a common pathway in co-morbidity development. Also, IBS-related depression and

anxiety are associated with malnutrition and microbiome alterations (12), which is also found in CD (21).

The microbiome is recently posited as an important player in homeostasis and pathogenesis (12). In this review, we focussed on the involvement on the gut-brain axis, but it is interesting to note that it that it could play a role in the pathogenesis of non-GI disorders too (109). One of these diseases is again RA, in which alterations in the microbiome are seen as an important environmental trigger in disease development (109). However, it can also provide a new mechanism for psychiatric co-morbidity (12). Whether microbiome alterations are a common feature for autoimmune diseases, is on this moment not known.

Depression and anxiety are complex disorders and the pathogenesis is multifactorial (106). Untangling the mechanisms that contribute to the development of depression and anxiety in CD is of pivotal importance for the development of new therapies. More focussed research on the effect of folate supplementation, probiotics and immune modulators on depression and anxiety in CD is recommended. For clinicians treating CD patients, it is important to be aware of the link between CD and depression and anxiety, in particular due to the increased suicide risk (33). Upon CD diagnosis, a psychiatric evaluation should be made in order to identify eventual psychiatric co-morbidity. The other way around, in patients with depressive and anxious symptoms, CD should be taken in consideration.

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