Neuro-Immuno-Gastroenterology

Cris S. Constantinescu
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Editors

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CSC would like to dedicate this book to his parents, who have inspired his interest in interdisciplinary science.
RA & VA dedicate this book to their son, Victor, their beacon of light and constant source of inspiration.

Preface

The multidirectional interaction between the immune system, the gut and the brain has only become subject of more intense research interest in the last few years. Much work has been done in neuroimmunology and in the immunology and immunopathogenesis of the gastrointestinal tract, and the influence of the nervous system on both immune functions (neuroimmunomodulation and nerve-driven immunity) and on gastrointestinal processes has been studied in detail. However, to date, no dedicated forum exists, either in the form of a scientific journal or in the form of a book, which addresses issues at the interface between the three biological disciplines, and in which the dialogue on specific common issues between neuroimmunologists and immuno-gastroenterologists can take place.

Recent discoveries in fundamental immunology require an intensification of this dialogue, so that understanding by scientists of one discipline of processes belonging to the other discipline is possible. For example, the fact that the gut has a critical function in shaping systemic immune responses, through innate lymphoid cells and mucosa-associated lymphoid tissues, means that scientists and physicians studying immune-mediated neurological disease need to gain insight into the immunology of the gastrointestinal system. Also, as it has recently become clear that the gut microbiota has a profound influence on the shaping of the immune response, as well as other processes such as metabolism, not only in the gastrointestinal system but also in the nervous system, scientists and clinicians in these fields require knowledge of the microbiology of the gastrointestinal tract. Fascinatingly, manipulation of the microbiota and of the intestinal immune responses can be used to modulate neurological and other immune-mediated inflammatory diseases.

Conversely, the nervous system and the psyche have significant effects on the functioning of the gastrointestinal tract.

Collaboration and communication between neuroscientists, gastrointestinal researchers, microbiologists and systematic biologists is essential for the advancement of this important field.

This book, an editorial collaboration between a neuroimmunologist and an immuno-gastroenterologist and nutrition scientist couple, is a first attempt at such a collaboration and communication. While not being an exhaustive, comprehensive

textbook on the subject, it is a collection of relevant topics that address many important issues regarding the interaction between the nervous system, the immune system and the digestive system in health and disease.

Introductory chapters into the immunology, the nervous system (enteric nervous system) and the microbiology of the gastrointestinal system, each written by experts in their research fields, provide necessary fundamentals of knowledge in these areas. The immunology chapter is written by gastroenterologists (Y Mahida and colleagues) with close attention to relevant disease entities. While not intended as a comprehensive review of the increasingly complicated landscape of the gut immune system, it provides essential information on this to allow the reader to put it in the context of immune-mediated inflammatory diseases. The chapter on the enteric nervous system, by a pathologist, M Constantinescu, in addition to a review of the anatomy of the nervous system of the gut, provides very useful information on the anatomic pathology of the enteric nervous system.

Important neurotransmitters, such as substance P (J Vilisaar and R Arsenescu) and vasoactive intestinal polypeptide (VIP, Ganea and colleagues), are discussed in particular with regard to their effect on the gut immune system and also their role in gut inflammation.

The role of stress in the gut-brain connection is expertly reviewed in a thorough, dedicated chapter by B Bonaz.

Five chapters deal with how gastrointestinal infections or commensal flora influence immune-mediated inflammatory diseases of the ventral and peripheral nervous system, while a separate chapter deals with the role of nutrition and macrobiotics in the gut and brain inflammation. In this context, the most widely discussed condition is the inflammatory demyelinating disease of the central nervous system, multiple sclerosis (MS), which, almost from its first clinicopathological descriptions by Charcot and his pupils, was postulated to have an infectious pathogenesis. Infections that potentially have a protective influence such as *Helicobacter pylori* (K Robinson, B Gran and colleagues) and intestinal parasites like *Necator americanus* (R Tanasescu) which is currently used in a phase II clinical trial of helminth immunotherapy in MS are thoroughly presented in separate chapters by established investigators in their respective areas, along with their potential immunomodulatory mechanisms in MS and its experimental model, experimental autoimmune encephalomyelitis (EAE). On the other hand, the negative influence of certain gut bacteria can be inferred from a thorough account of intestinal microbiota dysregulation in MS, with particular emphasis on Japanese MS (the epidemiology of which can be explained through major nutritional changes in Japan in the last decades), presented by a pioneering researcher in the field, T Yamamura, and by a comment on the role of certain bacteria and bacterial toxins in MS and neuromyelitis optica (NMO) by I-J Chou and CS Constantinescu. Nutrition and nutritional interventions, discussed in a chapter by V Arsenescu, for example, in the form of macrobiotics, are increasingly being studied in the context of inflammatory diseases of both the gastrointestinal and the nervous system. In the peripheral nervous system, N Shahrizaila and N Yuki use the best known example of molecular mimicry in autoimmunity, between *Campylobacter* and peripheral nerve glycolipids, to discuss the role of *Campylobacter jejuni* infection in the Guillain-Barré syndrome (GBS).

The coexistence between immune-mediated (autoimmune) inflammatory diseases of the nervous and the gastrointestinal system is another reason to support the development of neuro-immuno-gastroenterology. For example, MS is thought to coexist with inflammatory bowel disease more often than just by chance. Although this has been challenged by studies suggesting this association is merely due to the increased susceptibility of women to both of these conditions, the association seems to be genuine even after correcting for gender in some studies, suggesting common mechanisms of immunopathogenesis. On the other hand, even if the association were purely by chance, the frequency of the conditions means that management issues need considerations when they coexist, and not only common but differing immunopathogenesis mechanisms are to be taken into account. Demyelinating disease during or following treatment with anti-TNF agents for inflammatory bowel disease (and other immune-mediated diseases) is discussed in a chapter by SY Lim and CS Constantinescu. This is an unresolved issue, but the chapter provides a review of the information to date and balances the risk of coexistence of the conditions (with or without treatment) with that of "induction" of MS by anti-TNF treatment.

Cannabinoids are molecules involved in many biological functions, produced in large amounts in the central nervous system as endocannabinoids are fulfilling the definition of neurotransmitters (albeit nonclassical). The plant-derived or synthetic cannabinoids are also drugs that affect the immune, nervous system and gut functions. A team of pharmacologists led by P Gershkovich discuss novel ways of exploiting their physicochemical properties (liposolubility) to improve delivery to the gut lymphoid tissue to achieve and/or enhance neuroimmunological effects.

The last four chapters are dedicated to established neurological disease entities where the immune system and the gut play essential parts.

Gluten intolerance is an immune-mediated condition that is widely known to gastroenterologists. However, its neurological manifestations are less explored. Knowledge of these manifestations is important for gastroenterologists, nutritionists and neurologists alike. This is discussed in detail in the chapter by M Hadjivassiliou and colleagues. A view of pernicious anaemia, with interesting historical details, although the condition is certainly not a disease of the past, and in which often autoimmune mechanisms are critical, is provided by L Edwards. The profound effects in which the immune-mediated central nervous system disease, MS, affects the gut are expertly discussed by D Levinthal and K Bielefeldt. Finally, R Bassil and C Ionete review the neurological manifestation in the complex immunopathogenesis of the primary gastrointestinal condition of Whipple's disease.

The book does not aim to cover all aspects of neuro-immuno-gastroenterology. Functional neuro-gastroenterological disorders where inflammation or infection has been implicated, including irritable bowel syndrome and other motility disorders, are not discussed in a dedicated chapter. The mucosal immunology of the gut is an ever-increasing field as is the gut microbiota and its role in physiology and disease, so all very recent developments cannot be covered in a book like this.

However, the novelty of this book and the breadth of subjects covered offer a background of knowledge and an opening for the development of an important

interdisciplinary scientific and clinical area of research. We, therefore, hope that it will appeal to neurologists, gastroenterologists, nutritionists and other scientists interested in the complex interaction of the digestive, nervous and immune systems.

The idea of this book originated from many discussions between the editors and their colleagues, where the need for a more formalised interdisciplinary approach was recognised. We are grateful to all our colleagues involved in these fruitful discussions. We are also grateful to the Multiple Sclerosis Society of the UK and Northern Ireland, the funder of a large trial of helminth therapy in MS (the Worms for Immune Regulation in MS, or WIRMS Trial), which generated a number of the theoretical and practical questions that led to the idea of this book. We also are grateful for the readiness of *Springer* to consider and accept this novel idea.

The process of preparing and editing the multiauthored book is always slower than one wishes and at times frustrating, but the professionality, patience and editorial skills of the Springer editorial team of Joanna Bolesworth and Michael Griffin are gratefully acknowledged.

Nottingham, UK — Cris S. Constantinescu
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Chapter 1
The Immunology of the Gastrointestinal System

Emily Staples, Tanya M. Monaghan, and Yashwant Mahida

Abstract Distinctive features of the gastrointestinal immune system include its size, organisation and perpetual exposure to dietary antigens and a large and complex population of resident microorganisms. The mucosal immune system maintains homeostasis by avoiding tissue damaging responses to the luminal contents, whilst at the same time retaining the capacity to provide protection against pathogens. The luminal environment changes markedly from the proximal to the distal gastrointestinal tract, which may explain the restriction of many immune-mediated diseases to specific regions. Diseases that are considered in this chapter include pernicious anaemia, coeliac disease, those related to immunodeficiency, inflammatory bowel disease and bacterial infections that affect distinct regions of the gastrointestinal tract.

Keywords Stomach • Intestine • Autoimmunity • Infection • Inflammation

Organisation of the Mucosal Immune System

The gastrointestinal mucosal immune system is estimated to contain more lymphocytes than all the other peripheral lymphoid organs combined. Its unique features include the organisation of its lymphoid structures, characteristics of some of the lymphocyte subpopulations and constant exposure to dietary antigens, microorganisms (and their products) and other agents capable of modulating immune functions [1]. The majority of studies of the gastrointestinal immune system have involved the stomach, small intestine and colon. These regions have distinct luminal

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environments and may be affected by immune-mediated diseases that are often confined to the relevant parts of the gastrointestinal tract.

Secretion of gastric acid in the stomach provides protection against ingested pathogens, whereas the small intestine carries out the essential functions of digestion and absorption of nutrients. By contrast, large communities of bacteria are resident in the lumen of the large intestine where they are normally in a symbiotic relationship with the host [2]. The mucosal immune system of the gastrointestinal tract has the key role of maintaining normal homeostatic interactions by avoiding tissue damaging responses to not only the resident microbiota but also ingested antigens and immunomodulatory agents. In addition to providing protection against the resident microorganisms, the mucosal immune system is also capable of initiating inflammatory responses to invasive pathogens and their secreted products. At a cellular level, the above regions of the gastrointestinal tract are organised in distinct compartments that include (from luminal to the serosal surface) the mucosa, muscularis mucosa and submucosa. Cells of the mucosal immune system are predominantly located in the mucosa, in inductive and effector sites.

Aggregates of lymphocytes are prominent in distinct organised lymphoid structures, such as Peyer's patches in the small intestine, and they represent the main sites for the induction of intestinal adaptive immune responses. Specialised epithelial cells (designated microfold cells) on their surface enable the uptake and transport of luminal antigens to underlying antigen-presenting cells. Primed B and T cells exit the organised lymphoid structures via lymphatics and migrate via the mesenteric lymph nodes, thoracic duct and peripheral circulation, to home back to the effector compartment of the intestinal lamina propria, at sites distant from the initial antigen exposure [3]. The lymphocytes migrate from the peripheral circulation to the lamina propria via the adhesion molecules α4β7 integrin, which interacts with the mucosal addressin cell adhesion molecule 1 (MAdCAM-1) expressed on endothelial venules in the intestine [4]. Vitamin A, via its major active metabolite retinoic acid (which can be generated by dendritic cells and epithelial cells), has been shown to induce gut-homing properties of lymphocytes [5].

The majority of the immune cells in the effector compartment are present in the lamina propria of the mucosa, with smaller numbers of T cells (designated intraepithelial lymphocytes) in the overlying surface epithelium. In humans, the majority of the intraepithelial lymphocytes express αβ T-cell receptor (TCR) and a minor proportion are γδ TCR positive. In the lamina propria, there are more CD4+ than CD8+ mucosal T cells, and majority of them display an effector memory phenotype [1]. Subpopulations of the mucosal T cells include T helper (Th)1 cells, Th2 cells, regulatory T cells and Th17 cells. Large numbers of plasma cells are also present in the lamina propria, which mostly produce IgA1 or IgA2 that is transported by epithelial polymeric Ig receptor to the lumen (as secretory IgA). Mucosal innate lymphoid cells have recently been characterised, and their role in intestinal immunity and inflammation is of significant current interest [6]. Small populations of mucosal-associated invariant T cells (MAIT cells) and invariant natural killer cells (iNKT cells), which express invariant forms of the T cell receptor, have also been reported in the intestine [1]. It is of interest that MAITs have recently been implicated in

active lesions in the brain of patients with multiple sclerosis [7]. Macrophages and dendritic cells are prominent non-lymphoid cells in the lamina propria and demonstrate phenotypic and functional heterogeneity in the normal and inflamed intestine [8]. In the intestine, macrophages and dendritic cells are prominent under the surface epithelium where they can readily sample luminal antigens and also provide protection in the event of epithelial injury.

A single monolayer of epithelial cells lines the stomach and intestine and interacts closely with not only cells of the immune system but also luminal components. In the intestine, stem cells in epithelial crypts give rise to distinct subpopulations of the differentiated cells absorptive enterocytes, enteroendocrine cells, goblet cells and Paneth cells. The majority of the cells of the intestinal epithelium are replaced on a weekly basis, reflecting the dynamic nature of this compartment of the mucosa. Paneth cells, which are located at the crypt base, make an important contribution to innate immunity in the small intestine via their capacity to produce a number of antimicrobial peptides (such as alpha-defensins) and proteins (such as lysozyme) [9]. Mucin glycoproteins secreted by goblet cells provide a protective layer of mucus that lines the epithelial surface and consists of components of innate immunity (such as antimicrobial peptides) and adaptive immunity (secretory IgA).

Resident Microbial Flora

The gut microbiota has been described as an assortment of microorganisms inhabiting the length and width of the mammalian gastrointestinal tract. The composition of this complex microbial entity is host specific, evolving throughout an individual's lifetime, and is susceptible to both exogenous and endogenous modifications [10]. In humans, gut colonisation commences immediately after birth and then undergoes ecological succession with progressive environmental exposures, commensal interaction and various host factors [11, 12]. On average, 3 years after birth, the microbial community consists of a mixture of microbes that is largely similar to that found in the adult intestine [13]. The different phyla of bacteria in the microbiota are consistent from childhood to adulthood, but the species distribution is not constant due to various factors.

The intestinal microbial community comprises 70 % of the total microbiota found on the human body (total 10^{14} bacterial cells) and is highly diverse with well over 1000 bacterial species capable of colonising the human colon [14, 15]. It is estimated that the collective gene repertoire of the organisms comprising the gut microbiota contains well over 150 times more unique genes than the human genome [16]. The microbiota assembly and structure vary widely between different individuals and at different anatomical sites along the length of the intestinal tract [17, 18]. The microbiota of the large intestine is more dense and diverse than that seen in the small intestine [19, 20], and the bacterial taxa in these two sites differ [18, 20].

Likewise, the microbial populations associated with the mucus layer differ from those found in the intestinal lumen [10, 21].

The vast genomic dataset of the Human Microbiome Project has provided unparalleled insight into the composition, structure and temporal assembly of the microbiota [11]. In humans and mice, the major bacterial phyla that occupy the intestine during homeostasis are the *Firmicutes*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* [11]. Despite observed spatiotemporal differences in microbial composition, a health-associated microbiome is believed to be functionally conserved and contains a shared gene set necessary to perform important host physiological functions for the maintenance of human health [16]. These critical functions include aiding digestion of otherwise indigestible dietary polysaccharides into beneficial and absorbable short-chain fatty acids, synthesis of vitamins and other beneficial metabolites, degradation of xenobiotic substances, detoxification of potentially harmful substances such as bile acids and bilirubin, immune system regulation and enhanced resistance against colonisation by pathogenic microorganisms [18]. The precise mechanisms through which the microbiota exerts its beneficial or detrimental influences remain largely undefined, but include elaboration of signalling molecules and recognition of bacterial epitopes by both intestinal epithelial and mucosal immune cells [10].

Maturation of the intestinal mucosa and its immune system (including lymphoid structures such as Peyer's patches) is dependent upon colonisation by the microbiota [2, 22]. Using highly developed defence systems, in which the epithelium plays a critical role, the intestinal mucosa restricts the microbiota to the lumen. T cells, via subsets such as regulatory T cells and Th17 cells, maintain mutualistic interactions with the microbiota, whilst retaining the capacity to mediate host defences against microbial invasion [2, 22].

Autoimmune Gastritis/Pernicious Anaemia and Coeliac Disease

In autoimmune gastritis, chronic inflammation leads to loss of parietal cells and hypochlorhydria, which may progress to B12 malabsorption and pernicious anaemia (PA). The major target antigen is the H+/K+−ATPase located in the parietal cell canaliculi. This proton pump is recognised by pathogenic CD4+ T cells which recruit other inflammatory cells, including B cells which can secrete autoantibodies to the H+/K+−ATPase (parietal cell antibodies) and/or to intrinsic factor [23]. Parietal cell antibodies are found at high frequency, particularly early in the disease, though they may decline at later stages with parietal cell loss. They are not specific and may occur in other autoimmune conditions and in some healthy individuals, especially the elderly [24]. Autoantibodies to intrinsic factor (IF), a 60 kDa glycoprotein secreted by gastric parietal cells, are considered a more specific marker of PA. IF binds and transports vitamin B12 to the terminal ileum where it is absorbed. Two types of antibodies to IF

are described: type 1 antibodies block the B12 binding site; type 2 antibodies prevent absorption but are rarely seen in the absence of type 1 antibodies [24].

Autoimmune gastritis and *Helicobacter pylori* (*Hp*) can both cause gastric atrophy, but autoimmune gastritis typically affects the corpus with sparing of the antrum, whereas *Hp* infection usually results in more severe changes in the antrum [25]. Autoimmune gastritis and *Hp* infection may coexist. T-cell cloning studies identified some CD4+ T cells that proliferated and secreted IFN-γ in response to both *Hp* antigens and the H+/K+−ATPase, raising the possibility that *Hp* infection could trigger autoimmune gastritis via molecular mimicry [26]. *Hp* was detected more frequently in younger patients with PA [23]. Genetic factors also have a role as PA shows familial clustering and is associated with certain HLA-DR genotypes and with other autoimmune diseases, particularly autoimmune thyroid disease and type 1 diabetes [24].

Autoimmune gastritis can be asymptomatic if malabsorption is not a feature. Iron deficiency may occur as a low gastric pH is required for optimal iron absorption. Onset of anaemia may be insidious. In pernicious anaemia, there is macrocytic anaemia, low vitamin B12, atrophy of the gastric corpus and autoantibodies to intrinsic factor and/or gastric parietal cells. Prevalence is estimated to be approximately 2%, but this is difficult to ascertain reliably due to the complexity of the diagnosis and lack of biopsy data. Historically the Schilling test was used to assess B12 status, but this multistep test using radiolabelled vitamin B12 is now rarely performed. In addition to anaemia, deficiency of vitamin B12 can affect rapidly dividing cells leading to glossitis, diarrhoea and malabsorption. Ineffective erythropoiesis may lead to jaundice. Neurological abnormalities may start with demyelination, axonal degeneration and then neuronal death if not treated, which may manifest as peripheral neuropathy, weakness, ataxia or motor symptoms (subacute combined degeneration of the cord). A range of mental disturbances can also occur [24]. Treatment is with parenteral vitamin B12 with iron and folate replacement as required [27].

The chronic inflammation and further potential insults from infections able to colonise at the increased gastric pH make the stomach susceptible to development of hyperplastic and neoplastic lesions. Gastrin is secreted in response to the increased pH which can stimulate enterochromaffin-like cell hyperplasia and sometimes neuroendocrine tumours (formerly known as carcinoids), which are typically small with good prognosis [27]. PA has traditionally been considered a preneoplastic condition, but there is some controversy about the risk of development of gastric adenocarcinoma in this context as many of the studies were carried out prior to the discovery of *Hp*, and detection of current or previous *Hp* infection may be difficult in the context of atrophy [27].

Like autoimmune gastritis, coeliac disease is an autoimmune disease that occurs on a specific genetic background and involves mucosal pathology that is driven by CD4+ T cells with the development of autoantibodies. However, exogenous antigen in the form of dietary gluten is required for the development and maintenance of the small bowel enteropathy that occurs in coeliac disease. The vast majority of those with coeliac disease carry a particular variant of HLA-DQ2 or HLA-DQ8 [28, 29].

Prevalence is widely estimated to be as high as 1 %, although many cases remain undiagnosed which has led to the concept of the "coeliac iceberg". Gluten proteins have high glutamine and proline content, which makes them resistant to digestion, and include gliadins and glutenins in wheat, hordeins in barley and secalins in rye.

The autoantigen was identified as tissue transglutaminase (TTG) in 1997 [30]. This enzyme is able to modify neutral glutamine residues to negatively charged glutamate residues that have higher affinity for the HLA-DQ2/DQ8 binding pockets [31]. Antigen-presenting cells present these peptide-MHC II complexes to CD4+ T cells in the lamina propria, activating gluten-specific CD4+ T cells, which produce IFN-γ and IL-21 but not IL-17 or IL-22, and recruit and activate other lymphocytes [28, 31]. There are increased antigen-presenting cells and plasma cells in the lamina propria with expansion of TTG-specific IgA plasma cells [28, 31]. The transferrin receptor (CD71) can bind anti-gliadin secretory IgA facilitating the transport of intact peptide bound to IgA across the epithelial barrier to the lamina propria where it can prime more CD4+ T cells [31]. Increased frequencies of CD8+ T cells expressing the αβ and γδ TCRs occur in the epithelium of lesions from patients with coeliac disease, but it is the αβ TCR CD8+ intraepithelial lymphocytes that are thought to mediate most of the epithelial damage. They express NK receptors such as CD94 (NKG2C) and NKG2D that recognise the non-classical MHC class I molecules HLA-E and MICA, respectively, that are expressed by the intestinal epithelium in coeliac disease. Expression of IL-15 is upregulated by the epithelium, which acts as a co-stimulatory molecule for the TCR and NK cell receptors and disrupts oral tolerance by promoting proinflammatory dendritic cells. Gluten may have a direct effect by upregulating expression of IL-15 and non-classical MHC class I molecules by the stressed epithelium [28]. Dysregulated activity of the cytotoxic intraepithelial CD8+ T cells leads to destruction of intestinal epithelial cells and the typical histopathological findings of villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes.

Classic presenting features include diarrhoea, weight loss (or failure to thrive in children) and anaemia, but a broad range of gastrointestinal and extragastrointestinal symptoms and signs are now recognised [29]. The National Institute of Health and Clinical Excellence (NICE) recommends that coeliac testing is offered or considered in over 30 conditions, including irritable bowel syndrome, neuropathy and unexplained subfertility [32]. Population screening is not currently recommended, but serological testing should be offered to those with autoimmune thyroid disease, type 1 diabetes, dermatitis herpetiformis and first-degree relatives with coeliac disease due to the increased prevalence of coeliac disease in these groups [29, 32]. Risk of osteoporosis and bone fracture is increased, and coeliac disease is the most frequent cause of functional hyposplenism, which results in reduced immunity to encapsulated bacteria [29].

Diagnosis in adults is by a combination of serology and duodenal biopsy. Serological testing strategies vary between laboratories, but usually first-line testing is for IgA antibodies to the endomysium, TTG or deamidated gliadin, unless the patient is known to have IgA deficiency or low/absent IgA is detected. In this case, IgG-based serology may be undertaken, though this has lower sensitivity. Patients

with positive coeliac serology or negative serology and clinical suspicion of coeliac disease should be referred to a gastroenterologist for small bowel biopsies. Guidelines by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition propose that a diagnosis of coeliac disease may be made in symptomatic children without biopsies if they have TTG antibody levels greater than ten times the upper limit of normal, positive endomysial antibodies on a separate sample and the HLA-DQ2 or HLA-DQ8 genotypes associated with coeliac disease [33]. HLA typing is not used routinely in the diagnosis of coeliac disease in adults, but can help rule out coeliac disease in selected cases due to its high negative predictive value [29]. The mainstay of treatment is a gluten-free diet which leads to resolution of symptoms and histopathological and serological changes in most patients. Chronic inflammation increases the risk of T-cell lymphoma with those with persistent villous atrophy having the greatest risk [29].

Immunodeficiency and the Gastrointestinal Tract

As the gastrointestinal tract is the largest lymphoid organ in the body and acts as a barrier that must distinguish innocuous antigens such as food and commensal bacteria from pathogenic bacteria and toxins, it is not surprising that immunodeficiency or immune dysregulation often lead to gastrointestinal symptoms. Symptomatic primary immune deficiencies due to genetic defects are rare but often affect a specific part of the immune system providing an opportunity to assess its function. An increasing number of immunodeficiencies defined by single gene defects are being characterised [34]. Secondary immunodeficiency is more common and has many causes including malnutrition, infection, malignancy, drugs, protein loss, metabolic disease and toxins [35]. There is also increased susceptibility to infection at extremes of age. Several factors may combine in an individual to render them vulnerable to infection.

X-linked agammaglobulinaemia (XLA) was the first immunodeficiency to be described by Bruton in 1952. A defect in Bruton's tyrosine kinase (Btk) which is critical for the differentiation of pre-B cells into mature B cells is responsible. The classical immunological phenotype is low or absent B cells with all immunoglobulins low or absent. Presentation is typically around 6 months of age as levels of maternal IgG decline. Diarrhoea is often a feature and is most frequently due to *Giardia lamblia* and *Salmonella* or *Campylobacter* infection. Enteroviruses can also infect the gut and may cause chronic meningoencephalitis, though this is rare now as effective treatment with immunoglobulin replacement is standard. Stricturing and fissuring of the small bowel can occur, but no granulomas or plasma cells are seen on histology [36]. Tonsils are absent and there are no germinal centres in the gut-associated lymphoid tissue.

The commonest primary immune deficiency is selective IgA deficiency with estimated prevalence of 1 in 600. It is usually asymptomatic, but there is an increased risk of allergic and autoimmune disease, including coeliac disease. IgA-based coeliac

serology testing is unhelpful as IgA is undetectable in this condition. Gastrointestinal infections are surprisingly rare given that IgA is the main class of antibody involved in mucosal immunity. Giardiasis and the other gastrointestinal problems that occur in common variable immune deficiency (CVID) can also occur in selective IgA deficiency but less commonly [36]. In some cases, selective IgA deficiency can progress to CVID and there is a common MHC type. CVID is the commonest symptomatic primary immune deficiency (estimated prevalence, 1 in 25,000–50,000). It is a phenotypic diagnosis. Features include marked reduction in IgG and IgA and poor vaccine responses or low switched memory B cells [37]. This is a heterogeneous group of patients. The list of genes associated with subsets of patients with CVID continues to grow and includes genes involved in B-cell development and signalling (BAFF-R, TACI, CD19, CD20, CD21, CD81, PI3Kδ) but also genes involved in T-cell interactions and regulation (ICOS, CTLA-4) [34, 38, 39]. Infections are frequently caused by *Giardia lamblia*, *Salmonella* or *Campylobacter* species, but may also be due to organisms more in keeping with defective cell-mediated immunity such as CMV and *Cryptosporidium*. In the stomach a syndrome similar to pernicious anaemia with atrophic gastritis and malabsorption of vitamin B12 may occur, but antibodies to gastric parietal cells and intrinsic factor are absent. Screening for *Helicobacter pylori* has been proposed in view of the increased risk of gastric cancer in this population [40]. In the small bowel, villous atrophy or nodular lymphoid hyperplasia (NLH) can cause malabsorption. The villous atrophy that occurs in this context may mimic coeliac disease, but coeliac serology is negative, plasma cells are reduced or absent, the typical HLA genotypes are often not expressed and there is frequently no improvement on a gluten-free diet. In NLH multiple nodules are found in the lamina propria and/or submucosa. These contain large germinal centres and IgM^+ cells [36]. NLH may occur throughout the small intestine and occasionally in the stomach or colon. Other gastrointestinal complications include inflammatory bowel disease-like changes with microscopic or lymphocytic colitis.

Individuals with compromised cell-mediated immunity due to significantly reduced T-cell numbers or function are vulnerable to opportunistic intracellular infections with mycobacteria, viruses and fungi, including *Pneumocystis jirovecii*, in addition to bacterial pathogens. Examples of primary immune deficiencies with reduced T-cell-mediated immunity include X-linked hyper IgM syndrome and severe combined immunodeficiency (SCID). X-linked hyper IgM syndrome is due to mutations in CD40L (CD154). The lack of binding of CD40L on T cells to CD40 on B cells and other antigen-presenting cells impairs switching of IgM to IgG or IgA and antigen presentation to T cells. *Pneumocystis* pneumonia is a common presentation. Patients are advised to drink boiled water to reduce the risk of *Cryptosporidium* diarrhoea and monitored for ascending cholangitis and liver complications. SCID can be classified as T^-B^+ or T^-B^- depending whether B cells are present [34], but both groups have severe immune compromise as B cells are unable to function effectively in the absence of T cells. A number of genetic defects have been identified [34]. Infections usually begin early in life. Features in the gastrointestinal tract include chronic candidiasis and chronic diarrhoea, often secondary to viral infection [36]. If the diagnosis is suspected, urgent advice should be sought

from a paediatric immunologist. Early bone marrow transplant improves outcome which has led to introduction of newborn screening for SCID in some countries.

Mutations in the T regulatory cell gene *FOXP3* result in immune dysfunction polyendocrinopathy, enteropathy, X-linked (IPEX). This presents in the first few months of life with severe watery diarrhoea, demonstrating the key role of $FOXP3^+$ cells in the maintenance of the healthy gut mucosa. Autoantibodies against enterocytes, autoimmune enteropathy (AIE)-related 75 kDa antigen, have been reported [41].

Innate immune defects can also have profound effects on the gastrointestinal tract. For example, in chronic granulomatous disease (CGD), the neutrophil oxidative burst is defective due to mutations in components of the NADPH oxidase. The X-linked form where $gp91^{phox}$ is mutated is commonest, but autosomal recessive forms also occur. Patients with this condition are susceptible to infection with catalase-positive organisms such as *Staphylococcus aureus*, *Aspergillus*, *Nocardia* and *Serratia*, which may be deep seated, e.g. liver abscess and osteomyelitis. Gut involvement is common and may mimic Crohn's disease with involvement of any part of the length of the gastrointestinal tract and non-caseating granulomas. Large granulomas may cause obstruction.

The commonest cause of immunodeficiency worldwide is malnutrition due to lack of access to food or to chronic disease [35], though poor outcomes to infection in the malnourished are likely to be multifactorial. Studies in malnourished children have shown thymic atrophy and skewing of cytokines towards a Th2 response [42]. Antibody responses to vaccines are preserved in moderate malnutrition but become compromised in severe malnutrition. Intestinal barrier function is impaired [35, 42]. Studies suggest reduced chemotaxis and microbicidal activity of neutrophils and changes in intestinal flora compared to well-nourished children [42].

Infections themselves can cause immunodeficiency including viruses (EBV, CMV, measles, influenza) and acute and chronic bacterial infections. In most cases, there is transient lymphopaenia. HIV, however, causes chronic immune deficiency. Approximately 35 million people were living with the HIV virus at the end of 2013 [43]. The most affected region is sub-Saharan Africa, but it is important to consider and test for HIV infection in anyone with symptoms that could be consistent with HIV as highly effective treatment is available in the form of highly active antiretroviral therapy (HAART). HIV has tropism for $CD4^+$ cells, including T cells and macrophages. The gut-associated lymphoid tissue contains the majority of lymphocytes, and $CD4^+$ cells here become depleted in the acute phase of HIV infection along with $CD4^+$ cells in the peripheral blood. Intestinal $CD4^+$ T cells include regulatory T cells and Th17 cells which have roles in mucosal homeostasis and immunity. Depletion of these cells is thought to contribute to loss of intestinal barrier function with increased translocation of bacteria contributing to the systemic inflammatory response seen in HIV infection [44]. $CD4^+$ T cell help is required for optimal $CD8^+$ cytotoxic T-cell function, and T follicular helper cells are involved in priming humoral responses in germinal centre reactions.

Like patients with primary T-cell defects, HIV-infected patients with low $CD4^+$ T-cell counts are susceptible to infection by a wide variety of pathogens, though opportunistic infections are less frequently seen in the developed world since the

advent of HAART. In the upper GI tract candida, HSV or CMV may cause dysphagia. Causes of malabsorption and/or diarrhoea include crytosporidia, microsporidia, CMV and mycobacteria (including *Mycobacterium avium* complex). Kaposi's sarcoma secondary to HHV8 can occur anywhere along the GI tract and non-Hodgkin's lymphoma most frequently in the stomach. Anorectal disorders also occur, particularly in men who have sex with men. Proctitis may be the presentation of sexually transmitted infection with *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HSV, *Treponema pallidum* and CMV being typical pathogens. Non-opportunistic infections including bacteria, viruses and parasites also infect HIV-infected individuals more commonly. *C. difficile* is a frequent cause of diarrhoea in patients with HIV. Non-infective causes of symptoms should also be considered. Antiretrovirals may cause diarrhoea, particularly protease inhibitors. Idiopathic AIDS enteropathy is a diagnosis of exclusion [45].

In summary patients with immunodeficiency frequently develop gastrointestinal complications which may be infectious, autoimmune, inflammatory or malignant. The spectrum of likely pathogens will be determined by the immune defect. Atypical/recurrent presentation or lack of response to standard treatment should lead the physician to consider the possibility of immunodeficiency.

H. pylori Infection

Helicobacter pylori (*Hp*) is the commonest bacterium to infect the stomach. Its prevalence in developed countries has decreased in recent years, but it continues to colonise the stomachs of an estimated 70 % of the population in developing countries and 30–40 % in industrialised countries [46]. *Hp* stimulates innate and adaptive immune responses; however, infection and the resulting chronic inflammation usually persist life-long unless treated. The majority of those infected have asymptomatic chronic gastritis, but the damage associated with the persistent inflammation leads to peptic ulcer disease or gastric cancer in approximately 10–15 %. A number of bacterial virulence factors are associated with increased severity of inflammation and increased disease risk [47].

Innate defences include the barriers of the low pH of the stomach, the mucus layer and the epithelium. The virulence factor VacA can form pores in membranes allowing leakage of cell contents including urea, which acts as a substrate for urease, allowing *Hp* to buffer itself against the acid gastric environment. Most *Hp* is found in the mucus layer which is made up of glycosylated proteins known as mucins which can bind *Hp*, preventing it reaching the epithelium, for example, the blood group antigen Lewis b can bind *Hp* virulence factor BabA. This layer also acts as a matrix for antimicrobial peptides. Gastric epithelium consists of a sheet of polarised cells bound together by tight junctions. The *Hp* virulence factor CagA is able to associate with the tight junction scaffolding protein ZO-1 to disrupt the epithelial barrier. Epithelial cells secrete a number of cytokines, including IL-1β, IL-6

and IL-8, and express a number of toll-like receptors (TLRs), including TLR 4, TLR 5 and TLR 9. Immune cells in the gastric mucosa may also recognise *Hp* via TLRs and other pattern recognition receptors (PRRs), but the ligands for PRRs expressed by *Hp* appear to be less potent than those expressed by many other bacteria, which may aid immune evasion [47]. NOD1 recognises intracellular peptidoglycan triggering NF-κB activated proinflammatory pathways. This pathway is implicated in upregulation of β-defensin 2 by *Hp* [48]. This is probably the best studied antimicrobial peptide in the context of *Hp* infection, though others including β-defensin 4, α-defensins and LL-37 have also been found to be upregulated [47]. Relatively few studies have looked in detail at the cellular innate immune response, but neutrophils and macrophages infiltrate the *Hp*-infected gastric mucosa, and release of reactive oxygen species from these phagocytes is associated with tissue damage. In a subset of patients with severe gastritis or lymphoid follicles γδ, T cells were also increased [49]. Activated DCs have been identified in the gastric mucosa. Studies in mice indicate that Peyer's patches are an important site for induction of the adaptive immune response.

Mucosal and systemic IgG and IgA responses are mounted to *Hp*. There is some evidence that maternal anti-*Hp* IgA in breast milk can delay *Hp* colonisation in breastfed babies, but generally the antibodies seem to make little contribution to protective immunity. Serology is widely used to assess for current or recent *Hp* infection.

T cells form the largest component of the inflammatory cell infiltrate in *Hp*-associated gastritis, with an increase in CD4:CD8 T cell ratio compared to the uninfected gastric mucosa. In mouse models, αβ T cells were required for control of *Helicobacter* infection and development of precancerous changes [50]. MHC II-deficient mice lacked protection in mouse vaccination studies, and transfer of $CD4^+$ T cells to T-cell-deficient mice restored preneoplastic pathology [50]. IFN-γ-secreting Th1 cells are increased in *Hp* infection and are associated with inflammation in both *Helicobacter*-infected humans and animals [51]. Th17 responses were associated with neutrophil recruitment and protection in a mouse vaccination model [52], and IL-17 expression also correlated with neutrophil infiltration in patients [53]. Lower Th17 and higher regulatory T-cell frequencies were found in children, in keeping with the reduced *Hp*-associated inflammation and pathology typically found in children [53]. Mice and humans with low frequencies of regulatory T cells had lower density of *Hp* colonisation with more severe gastritis, with increased risk of peptic ulcer disease in the humans [47]. There is also a systemic regulatory T-cell response to *Hp* which may protect against allergy (see Chap. 6). Frequencies of Th2 cells are increased in the *Hp*-infected gastric mucosa [47]. IL-$4^{-/-}$ mice had more severe gastritis and higher levels of IFN-γ, suggesting a possible protective effect. In some patients, the ongoing inflammatory response to *Hp* leads to the development of the precancerous changes of atrophy and/or intestinal metaplasia. Other primary gastric infections are rare, but when atrophy occurs, with loss of the acid-secreting glands, the stomach pH increases and superinfection with other bacteria may occur.

Enterovirulent Bacteria and Immunopathogenesis

Enterovirulent bacteria colonise various sites in the human intestine. *Vibrio cholerae*, *Salmonella enterica* serovar Typhi, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and diffusely adherent *E. coli* (DAEC) preferentially affect the small intestine, whereas *Shigella* spp., *Campylobacter* spp., enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) and toxigenic *C. difficile* infect the colon. *Yersinia* spp., enteroaggregative *E. coli* (EAEC) and *Salmonella* spp. affect both the small and large intestines [54].

Human intestinal bacterial pathogens are equipped with a variety of sophisticated weapons that provide them with mechanisms for subverting the cellular machinery and circumventing host innate and adaptive immune responses. Immunologists have frequently turned to *Salmonella* infection models to expand understanding of host immunity to intestinal pathogens.

Salmonella Infection

Salmonella enterica is a flagellated, Gram-negative, facultative intracellular bacterial species that is the leading cause of enteric disease in humans and animal hosts. Infection manifests itself through a broad range of clinical symptoms and can result in asymptomatic carriage, gastroenteritis, systemic disease such as typhoid fever and, in severe cases, death [55]. The variations in the clinical features of infection with this intracellular pathogen relate to differences in the interaction between different *Salmonella* serovars and the host. Although about 90% of the genes in *S. typhi* and *S. typhimurium* serovars are identical, more that 200 of the 4000 *S. typhi* genes are functionally disrupted or inactive [56]. This could in part explain the different immune responses both serovars induce upon entering their host [57]. *S. typhimurium* and *S. typhi* express various virulence factors including the type III secretion (T3SS) system (secretes effector proteins into the target cell cytosol, which manipulate host cell signalling cascades), lipopolysaccharide and other surface polysaccharides, fimbrae, flagellin and bacterial DNA that are essential for the intracellular lifecycle of *S. enterica* [57]. Genes for these factors are commonly carried on the *Salmonella* pathogenicity islands (SPIs) [58, 59].

Following adherence to epithelial cells in the gut, *Salmonella* targets antigen-sampling microfold (M) cells overlying Peyer's patches to translocate across the gut epithelium [60]. Indeed, M cells can transport a diverse array of mucosal enteropathogens across the intestinal epithelial barrier, including *Vibrio cholerae*, *Campylobacter jejuni*, *Shigella* spp., *Escherichia coli* and *Yersinia* spp. [61]. In fact, alongside Salmonella species, *Shigella* spp. and *Yersinia* spp. are capable of directly invading and destroying M cells and spreading the infection to neighbouring enterocytes [61]. *Salmonella* may also induce its internalisation in non-phagocytic enterocytes through its virulence-associated T3SS encoded by SPI-1 [62]. In

addition, invasion also has been proposed to occur by paracellular pathways following disruption of tight junctions or via CX3CR1-expressing macrophages/dendritic cells, which intercalate between epithelial cells [63, 64]. When internalised, bacteria reside within the cell cytoplasm within large vesicles called *Salmonella*-containing vacuoles (SCVs), where they replicate. The SCVs then transcytose to the basolateral membrane and release the bacteria to the submucosa, in which they are internalised within resident phagocytes and maintained by a second T3SS, encoded on a second pathogenicity island, SPI-2 [54]. SPI-2 promotes protection from reactive oxygen intermediates produced by macrophages, specifically nitric oxide (NO) and NADPH oxidase [65, 66]. The detection of *Salmonella* by TLRs has been shown to be crucial for Salmonella virulence, since it induces the acidification of the intramacrophage phagosome which in turn provides a cue for *Salmonella* that it has reached its intracellular niche protected from extracellular immune responses [67]. Whilst non-typhoidal strains remain restricted to the GI tract, typhoidal *Salmonella* serovars then disseminate through the lymph and bloodstream to the mesenteric lymph nodes and colonise systemic sites, such as the liver and spleen [68].

Following invasion of the intestinal mucosa, *Salmonella*-derived ligands are detected by a multitude of PRRs which include NOD-like receptors and TLRs, inducing a transcriptional response leading to the expression of key cytokines such as IL-18 and IL-23, which amplify the inflammatory response by paracrine signalling mechanisms, inducing the massive secretion of IFN-γ, IL-22 and IL-17 by mucosa-resident T cells [68]. These cytokines induce the increased production of mucins and antimicrobial peptides and promote the release of CXC chemokines leading to an influx of neutrophils into the mucosa [68]. Recent reports indicate that *S. typhimurium* exploits intestinal mucosal inflammation to outcompete the microbiota and thus increase its growth in the lumen of the inflamed gut. Mechanistically, this pathogen induces host-driven production of reactive oxygen species that generate a novel respiratory electron acceptor, which can be used by *Salmonella* but not the microbiota [69]. Thus, the ability to trigger intestinal inflammation is crucial for *S. typhimurium* to overgrow other microbes in the gut. Another recent study suggests that *Salmonella* exploits IL-22 host defences to control their growth [70]. Normally IL-22 binds to receptors on colonocytes and promotes production of antimicrobial molecules including lipocalin and two subunits of calprotectin. Lipocalin and calprotectin bind metal ions, which are essential for bacterial replication. However, Salmonella expresses proteins (salmochelin and ZnuABC) that can steal metal ions from lipocalin and calprotectin and thus successfully outcompete its nearest commensal neighbours, *E. coli* and other gut flora. In IL-22-deficient mice, there are fewer antimicrobial factors expressed, and both *Salmonella* and *E. coli* colonise the gut [70, 71].

Flagellin injected into host cells by invading *S. typhimurium* induces inflammasome activation through NLRC4, a member of the nucleotide binding domain leucine-rich repeat (NLR) protein family that responds to cytosolic bacterial products [72]. During systemic infection, Salmonella avoids NLRC4 inflammasome

activation by down-regulating flagellin expression [73, 74]. Furthermore, activation of the NLRC4 inflammasome occurs as part of the innate immune response during infections with *Yersinia* and *Shigella* species [75, 76].

In terms of adaptive immunity, there is good evidence that murine *Salmonella* infection induces the expansion of antigen-specific CD4 T cells in secondary lymphoid tissues and may have a role in clearing primary infection as well as also being required for acquired resistance to secondary infection [77, 78]. Activated CD4 T cells acquire the ability to home to sites of infection and produce IFN-γ to activate infected macrophages [79]. Recent studies also suggest that protective immunity may be conferred by regulatory T cells which modulate the potency of *Salmonella*-specific Th1 cells *in vivo* and Th17 cells [79]. In contrast, B cells are dispensible for resolving primary Salmonella infection but are required for protection against secondary challenge [80–82]. However, the mechanism by which B cells contribute to protective immunity against *Salmonella* remains unclear. It is postulated that antibody has direct access to *Salmonella* after phagocyte cell death when *Salmonella* are presumably found in the extracellular compartment. In this regard, opsonisation of bacteria with Salmonella-specific antibody impedes bacterial colonisation *in vivo* [83]. However, susceptibility to typhoid infection has been reported to occur despite the presence of elevated titres of antibodies against O, H, and other *S. typhi* antigens [84]. For more detailed discussions on humoral and cell-mediated immunity to *S. typhi* and *S. paratyphi* acquired through natural infection, experimental challenge and vaccination, the reader is referred to an excellent recently published review [84].

Other Type III Secretion System Effectors

Alongside *Salmonella*, several other enteric bacteria such as EPEC and EHEC, *Shigella* and *Yersinia* species, use T3SS effectors to facilitate their own attachment or invasion, subvert endocytic trafficking, block phagocytosis, modulate apoptotic pathways and manipulate innate immunity and host responses as part of the initial infection process. These are extensively reviewed elsewhere [85].

Campylobacter jejuni *Infection*

The food-borne pathogen, *Campylobacter jejuni,* is a Gram-negative, microaerophilic, spiral-shaped and motile bacterium, which is the most prevalent cause of bacterial gastroenteritis worldwide [86, 87]. Bacterial factors implicated in host cell invasion and disease pathogenesis include capsular polysaccharide, adhesive factors, flagellar apparatus, cytolethal distending toxin and post-translational glycosylation [88, 89]. Despite increasing knowledge of the role of these virulence-associated factors in disease pathogenesis, the mechanisms and consequences of the host

immune response to *C. jejuni* infection remain unclear, particularly with respect to its role in the development of inflammatory disease.

It is well established that *Campylobacter* cells are able to invade and translocate intestinal epithelial layers both *in vivo* and *in vitro* in the absence of T3SS, but the mechanisms that control cell entry are not fully understood [54]. Human intestinal epithelial cell transcriptional regulation and secretion of antimicrobials (β-defensin) and chemokines (IL-8, monocyte chemoattractant protein 1 and macrophage inflammatory protein 1β) have been proposed to play a role in *C. jejuni*-mediated intestinal inflammation [90–92]. Currently, it is believed that *C. jejuni* stimulates innate immune responses through activation of NF-κB signalling pathways via the mitogen-activated protein kinase family [93]. Activation of these pathways occurs secondary to binding of bacterial cell wall compounds to NOD or to TLRs. Evidence links the cytosolic NOD1 receptor in the host cell recognition of *C. jejuni* cell components and IL-8 signalling [94]. In addition, *C. jejuni* surface polysaccharides induce IL-6 secretion from intestinal epithelial cells via TLR2 in a MyD88-independent manner [95]. Moreover, an important role for TLR4 signalling in *C. jejuni* immunopathology has been confirmed in murine models of disease [96, 97].

Further, NF-κB's capacity to stimulate various cytokines in turn mediates maturation of dendritic cells into antigen-presenting cells, which shape subsequent B- and T-cell responses. Corresponding IgA and IgG antibodies produced by mature B cells against *C. jejuni* are considered to contribute to long-term protection against reinfection, but they might be detrimental when cross-reacting with gangliosides in neurons which in turn results in neurological sequelae such as Guillain-Barré syndrome in about 1 in 900 infected patients [97]. *C. jejuni* also induces adaptive intestinal T-cell responses in *ex vivo* infected explants of infected human colon tissue stimulating the release of IFN-γ, IL-22, IL-17A, IL-12, IL-23, IL-1β and IL-6 from neutrophils, macrophages and dendritic cells [98]. In addition to their known antimicrobial functions, IL-17 family members reduced the number of intracellular *C. jejuni* in intestinal epithelia [98].

Clostridium difficile *Infection*

C. difficile is a Gram-positive rod-shaped and toxin-producing bacterium that is capable of forming highly resistant endospores that facilitate its transmission. Following the loss of protection mediated by resident bacteria, *C. difficile* spores can germinate and grow as vegetative bacteria, resulting in the development of disease that ranges from mild diarrhoea to colitis and toxin megacolon [99, 100]. At endoscopy, characteristic pseudomembranes may be seen, which are due to focal areas of inflammatory exudate. Colonic inflammation is mediated by two secreted toxins, toxins A and B, that after uptake inactivate the Rho family of GTPases by glycosylation, with subsequent disruption of the cell cytoskeleton [100, 101]. Inhibition of these critical signalling molecules leads to actin cytoskeleton disruption, intestinal epithelial cell damage and apoptosis by caspase activation.

Toxins that gain access to the epithelium initially induce loss of barrier function and expression of cytokines, with subsequent cell death by apoptosis [100]. In addition to induction of IL-8 secretion by epithelial cells, innate immune responses induced include caspase 1 inflammasome activation via pyrin [102]. Pattern recognition receptors (PRRs), such as NOD1 and TLR 2, TLR 4 and TLR 5, have also been implicated in disease pathogenesis [100].

A number of studies suggest that humoral immune responses to toxins A and B may determine the nature of clinical presentation following colonisation with toxigenic *C. difficile* [100]. Thus, high serum concentrations of antitoxin antibodies have been associated with asymptomatic carriage, whereas low antibody levels have been reported in those with recurrent disease. The role of cell wall-associated antigens, which may be involved in bacterial adhesion to epithelial cells, is also currently under active investigation.

Inflammatory Bowel Disease

Inflammatory bowel disease consists of two chronic inflammatory conditions, ulcerative colitis and Crohn's disease. Prevalence of the two diseases is approximately similar in Europe and North America, together affecting about 400 individuals per 100,000 population [103]. These diseases have distinct and shared clinical and histological features. Ulcerative colitis only affects the colon, with continuous mucosal inflammation extending for variable distances proximally from the rectum. Crohn's disease can involve any part of the gastrointestinal tract, but the small and large intestine are the commonest regions affected. In contrast to ulcerative colitis, chronic inflammation in Crohn's disease may occur in distinct segments of the intestine (skip lesions), often with largely uninvolved intervening mucosa. Inflammation is confined to the mucosa in ulcerative colitis, but often affects the whole thickness of the intestinal wall in Crohn's disease, in which non-caseating granulomas are a characteristic feature in many affected individuals. Moreover, segments of the intestine may be affected by fibrosis and strictures in Crohn's disease, often requiring surgical resection. The aetiopathogenesis of ulcerative colitis and Crohn's disease remains to be fully understood, but studies to date suggest that they arise due to inappropriate immunological and inflammatory responses to luminal microorganisms in genetically susceptible individuals [104, 105]. Genome-wide association scans have identified more than 150 inflammatory bowel disease susceptibility loci, and most contribute to both diseases but are believed to explain only a minority of the variance in disease risk [106]. Possible causal genes suggest a major role for interactions between the host mucosal immune system and microorganisms, in which some of the commensal microbiota change their association with the host from a symbiotic to a pathogenic relationship. Some of the genetic associations are shared with other autoimmune diseases, for example, between IL23 receptor gene and inflammatory bowel disease, psoriasis and ankylosing spondylitis. NOD2 was the first gene that was reported to be associated with Crohn's disease, and its product is an intracellular sensor of bacterial peptidoglycan. Homozygosity/

compound heterozygosity for one and/or other of the three polymorphisms (which impair responses to peptidoglycan) confers an 11–27-fold increased risk of Crohn's disease, which is the highest relative risk observed for any of the genes associated with this disease [105]. Studies have shown that following recognition of bacterial peptidoglycan, NOD2 influences innate and adaptive immune responses via expression in cells such as Paneth cells (which express antimicrobial peptides and proteins), macrophages and dendritic cells. Additional biological functions that may be affected by polymorphisms in other genes associated with inflammatory bowel disease include epithelial barrier function, autophagy and cell migration [104].

Investigation of changes in the microbiota in inflammatory bowel disease is of significant current interest [100]. Reports include reduction in the variety of bacterial species and decrease in some dominant commensal members. It is possible that some of the changes to the microbiota are secondary to the inflammatory response, rather than being causal.

For moderate to severely active inflammatory bowel disease, anti-inflammatory agents that target cells of the immune system are widely used. They include corticosteroids and monoclonal antibodies that target tumour necrosis factor and, more recently, adhesion molecules on gut-homing lymphocytes [107]. There has also been recent interest in the role of autologous haematopoietic stem cell transplantation in the treatment of patients with severe Crohn's disease that is refractory to standard to medical treatment [108].

References

1. Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol. 2014;14:667–85.
2. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. 2010;10:159–69.
3. Macpherson A, Maloy K. Adaptive immunity in the gastrointestinal tract. In: Mahida YR, editor. Immunological aspects of gastroenterology. Dordrecht/Boston/London: Kluwer Academic Publishers; 2001. p. 35–51.
4. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76:301–14.
5. Veldhoen M, Brucklacher-Waldert V. Dietary influences on intestinal immunity. Nat Rev Immunol. 2012;12:696–708.
6. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells – a proposal for uniform nomenclature. Nat Rev Immunol. 2013;13:145–9.
7. Abrahamsson SV, Angelini DF, Dubinsky AN, et al. Non-myeloablative autologous haematopoietic stem cell transplantation expands regulatory cells and depletes IL-17 producing mucosal-associated invariant T cells in multiple sclerosis. Brain. 2013;136:2888–903.
8. Mahida YR. The key role of macrophages in the immunopathogenesis of inflammatory bowel disease. Inflamm Bowel Dis. 2000;6:21–33.
9. Elphick DA, Mahida YR. Paneth cells: their role in innate immunity and inflammatory disease. Gut. 2005;54:1802–9.
10. Sekirov I, Russell SL, Antunes LC, et al. Gut microbiota in health and disease. Physiol Rev. 2010;90:859–904.

11. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486:207–14.
12. Human Microbiome Project C. A framework for human microbiome research. Nature. 2012;486:215–21.
13. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.
14. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol. 1977;31:107–33.
15. Rajilic-Stojanovic M, Smidt H, de Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. Environ Microbiol. 2007;9:2125–36.
16. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65.
17. Lozupone CA, Stombaugh J, Gonzalez A, et al. Meta-analyses of studies of the human microbiota. Genome Res. 2013;23:1704–14.
18. Walker AW, Lawley TD. Therapeutic modulation of intestinal dysbiosis. Pharmacol Res. 2013;69:75–86.
19. Garner CD, Antonopoulos DA, Wagner B, et al. Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a Salmonella enterica serovar typhimurium murine model of infection. Infect Immun. 2009;77:2691–702.
20. Ubeda C, Taur Y, Jenq RR, et al. Vancomycin-resistant enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. J Clin Invest. 2010;120:4332–41.
21. Swidsinski A, Loening-Baucke V, Lochs H, et al. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World J Gastroenterol. 2005;11:1131–40.
22. Maynard CL, Elson CO, Hatton RD, et al. Reciprocal interactions of the intestinal microbiota and immune system. Nature. 2012;489:231–41.
23. Varbanova M, Frauenschlager K, Malfertheiner P. Chronic gastritis – an update. Best Pract Res Clin Gastroenterol. 2014;28:1031–42.
24. Bizzaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmun Rev. 2014;13:565–8.
25. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996;20:1161–81.
26. Amedei A, Bergman MP, Appelmelk BJ, et al. Molecular mimicry between Helicobacter pylori antigens and H+, K+−-adenosine triphosphatase in human gastric autoimmunity. J Exp Med. 2003;198:1147–56.
27. Neumann WL, Coss E, Rugge M, et al. Autoimmune atrophic gastritis – pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol. 2013;10:529–41.
28. Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nat Rev Immunol. 2013;13:294–302.
29. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 2014;63:1210–28.
30. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med. 1997;3:797–801.
31. Meresse B, Malamut G, Cerf-Bensussan N. Celiac disease: an immunological jigsaw. Immunity. 2012;36:907–19.
32. NICE. Coeliac disease: recognition and assessment of coeliac disease. 2009.
33. Husby S, Koletzko S, Korponay-Szabo IR, et al. European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54:136–60.
34. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol. 2014;5:162.

35. Chinen J, Shearer WT. Secondary immunodeficiencies, including HIV infection. J Allergy Clin Immunol. 2010;125:S195–203.
36. Agarwal S, Mayer L. Diagnosis and treatment of gastrointestinal disorders in patients with primary immunodeficiency. Clin Gastroenterol Hepatol. 2013;11:1050–63.
37. ESID. ESID registry – working definitions for clinical diagnosis of PID. 2014.
38. Angulo I, Vadas O, Garcon F, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. Science. 2013;342:866–71.
39. Schubert D, Bode C, Kenefeck R, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med. 2014;20:1410–6.
40. Dhalla F, da Silva SP, Lucas M, et al. Review of gastric cancer risk factors in patients with common variable immunodeficiency disorders, resulting in a proposal for a surveillance programme. Clin Exp Immunol. 2011;165:1–7.
41. Kobayashi I, Kubota M, Yamada M, et al. Autoantibodies to villin occur frequently in IPEX, a severe immune dysregulation, syndrome caused by mutation of FOXP3. Clin Immunol. 2011;141:83–9.
42. Rytter MJ, Kolte L, Briend A, et al. The immune system in children with malnutrition – a systematic review. PLoS One. 2014;9, e105017.
43. WHO. WHO data and statistics – HIV: global situation and trends. 2014.
44. Xu H, Wang X, Veazey RS. Mucosal immunology of HIV infection. Immunol Rev. 2013;254:10–33.
45. Vachon M-LC, Dieterich DT. Gastrointestinal disorders in HIV including diarrhoea. In: Volberding PA, Greene WC, Lange J, Gallant J, Sewankambo N, editors. Sande's HIV/AIDS medicine: medical management of AIDS 2012: Elsevier; 2012.
46. Barzilay EJ, Fagan RP. Helicobacter pylori. 2012. http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/helicobacter-pylori.htm. Accessed 20 Jan 2013: Centres for Disease Control and Prevention. Chapter 3: Infectious diseases related to travel.
47. Robinson K, Argent RH, Atherton JC. The inflammatory and immune response to Helicobacter pylori infection. Best Pract Res Clin Gastroenterol. 2007;21:237–59.
48. Patel SR, Smith K, Letley DP, et al. Helicobacter pylori downregulates expression of human beta-defensin 1 in the gastric mucosa in a type IV secretion-dependent fashion. Cell Microbiol. 2013;15:2080–92.
49. Futagami S, Hiratsuka T, Suzuki K, et al. Gammadelta T cells increase with gastric mucosal interleukin (IL)-7, IL-1beta, and Helicobacter pylori urease specific immunoglobulin levels via CCR2 upregulation in Helicobacter pylori gastritis. J Gastroenterol Hepatol. 2006;21:32–40.
50. Hitzler I, Kohler E, Engler DB, et al. The role of Th cell subsets in the control of Helicobacter infections and in T cell-driven gastric immunopathology. Front Immunol. 2012;3:142.
51. Robinson K, Kenefeck R, Pidgeon EL, et al. Helicobacter pylori-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. Gut. 2008;57:1375–85.
52. Velin D, Favre L, Bernasconi E, et al. Interleukin-17 is a critical mediator of vaccine-induced reduction of Helicobacter infection in the mouse model. Gastroenterology. 2009;136:2237–46. e1.
53. Serrano C, Wright SW, Bimczok D, et al. Downregulated Th17 responses are associated with reduced gastritis in Helicobacter pylori-infected children. Mucosal Immunol. 2013;6:950–9.
54. Lievin-Le Moal V, Servin AL. Pathogenesis of human enterovirulent bacteria: lessons from cultured, fully differentiated human colon cancer cell lines. Microbiol Mol Biol Rev. 2013;77:380–439.
55. Hurley D, McCusker MP, Fanning S, et al. Salmonella-host interactions – modulation of the host innate immune system. Front Immunol. 2014;5:481.
56. McClelland M, Sanderson KE, Spieth J, et al. Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature. 2001;413:852–6.
57. de Jong HK, Parry CM, van der Poll T, et al. Host-pathogen interaction in invasive Salmonellosis. PLoS Pathog. 2012;8, e1002933.

58. Valdez Y, Ferreira RB, Finlay BB. Molecular mechanisms of Salmonella virulence and host resistance. Curr Top Microbiol Immunol. 2009;337:93–127.
59. Fabrega A, Vila J. Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clin Microbiol Rev. 2013;26:308–41.
60. Jones BD, Ghori N, Falkow S. Salmonella typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. J Exp Med. 1994;180:15–23.
61. Miller H, Zhang J, Kuolee R, et al. Intestinal M cells: the fallible sentinels? World J Gastroenterol. 2007;13:1477–86.
62. Francis CL, Starnbach MN, Falkow S. Morphological and cytoskeletal changes in epithelial cells occur immediately upon interaction with Salmonella typhimurium grown under low-oxygen conditions. Mol Microbiol. 1992;6:3077–87.
63. Vazquez-Torres A, Jones-Carson J, Baumler AJ, et al. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. Nature. 1999;401:804–8.
64. Niess JH, Brand S, Gu X, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science. 2005;307:254–8.
65. Chakravortty D, Hensel M. Inducible nitric oxide synthase and control of intracellular bacterial pathogens. Microbes Infect. 2003;5:621–7.
66. Vazquez-Torres A, Xu Y, Jones-Carson J, et al. Salmonella pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science. 2000;287:1655–8.
67. Arpaia N, Godec J, Lau L, et al. TLR signaling is required for Salmonella typhimurium virulence. Cell. 2011;144:675–88.
68. Broz P, Ohlson MB, Monack DM. Innate immune response to Salmonella typhimurium, a model enteric pathogen. Gut Microbes. 2012;3:62–70.
69. Winter SE, Thiennimitr P, Winter MG, et al. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature. 2010;467:426–9.
70. Behnsen J, Jellbauer S, Wong CP, et al. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. Immunity. 2014;40:262–73.
71. Monack DM. The battle in the gut. Immunity. 2014;40:173–5.
72. Franchi L, Kamada N, Nakamura Y, et al. NLRC4-driven production of IL-1beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. Nat Immunol. 2012;13:449–56.
73. Cummings LA, Barrett SL, Wilkerson WD, et al. FliC-specific CD4+ T cell responses are restricted by bacterial regulation of antigen expression. J Immunol. 2005;174:7929–38.
74. Cummings LA, Wilkerson WD, Bergsbaken T, et al. In vivo, fliC expression by Salmonella enterica serovar Typhimurium is heterogeneous, regulated by ClpX, and anatomically restricted. Mol Microbiol. 2006;61:795–809.
75. von Moltke J, Ayres JS, Kofoed EM, et al. Recognition of bacteria by inflammasomes. Annu Rev Immunol. 2013;31:73–106.
76. Koizumi Y, Toma C, Higa N, et al. Inflammasome activation via intracellular NLRs triggered by bacterial infection. Cell Microbiol. 2012;14:149–54.
77. Nauciel C. Role of CD4+ T cells and T-independent mechanisms in acquired resistance to Salmonella typhimurium infection. J Immunol. 1990;145:1265–9.
78. Hess J, Ladel C, Miko D, et al. Salmonella typhimurium aroA- infection in gene-targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN-gamma in bacterial clearance independent of intracellular location. J Immunol. 1996;156:3321–6.
79. Griffin AJ, McSorley SJ. Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. Mucosal Immunol. 2011;4:371–82.
80. Mastroeni P, Simmons C, Fowler R, et al. Igh-6(−/−) (B-cell-deficient) mice fail to mount solid acquired resistance to oral challenge with virulent Salmonella enterica serovar typhimurium and show impaired Th1 T-cell responses to Salmonella antigens. Infect Immun. 2000;68:46–53.

81. McSorley SJ, Jenkins MK. Antibody is required for protection against virulent but not attenuated Salmonella enterica serovar typhimurium. Infect Immun. 2000;68:3344–8.
82. Mittrucker HW, Raupach B, Kohler A, et al. Cutting edge: role of B lymphocytes in protective immunity against Salmonella typhimurium infection. J Immunol. 2000;164:1648–52.
83. Cunningham AF, Gaspal F, Serre K, et al. Salmonella induces a switched antibody response without germinal centers that impedes the extracellular spread of infection. J Immunol. 2007;178:6200–7.
84. Sztein MB, Salerno-Goncalves R, McArthur MA. Complex adaptive immunity to enteric fevers in humans: lessons learned and the path forward. Front Immunol. 2014;5:516.
85. Raymond B, Young JC, Pallett M, et al. Subversion of trafficking, apoptosis, and innate immunity by type III secretion system effectors. Trends Microbiol. 2013;21:430–41.
86. Young KT, Davis LM, Dirita VJ. Campylobacter jejuni: molecular biology and pathogenesis. Nat Rev Microbiol. 2007;5:665–79.
87. Poly F, Guerry P. Pathogenesis of Campylobacter. Curr Opin Gastroenterol. 2008;24:27–31.
88. Zilbauer M, Dorrell N, Wren BW, et al. Campylobacter jejuni-mediated disease pathogenesis: an update. Trans R Soc Trop Med Hyg. 2008;102:123–9.
89. Rubinchik S, Seddon A, Karlyshev AV. Molecular mechanisms and biological role of Campylobacter jejuni attachment to host cells. Eur J Microbiol Immunol (Bp). 2012;2:32–40.
90. Bakhiet M, Al-Salloom FS, Qareiballa A, et al. Induction of alpha and beta chemokines by intestinal epithelial cells stimulated with Campylobacter jejuni. J Infect. 2004;48:236–44.
91. Hu L, Hickey TE. Campylobacter jejuni induces secretion of proinflammatory chemokines from human intestinal epithelial cells. Infect Immun. 2005;73:4437–40.
92. Zilbauer M, Dorrell N, Boughan PK, et al. Intestinal innate immunity to Campylobacter jejuni results in induction of bactericidal human beta-defensins 2 and 3. Infect Immun. 2005;73:7281–9.
93. Watson RO, Galan JE. Signal transduction in Campylobacter jejuni-induced cytokine production. Cell Microbiol. 2005;7:655–65.
94. Zilbauer M, Dorrell N, Elmi A, et al. A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to Campylobacter jejuni. Cell Microbiol. 2007;9:2404–16.
95. Friis LM, Keelan M, Taylor DE. Campylobacter jejuni drives MyD88-independent interleukin-6 secretion via Toll-like receptor 2. Infect Immun. 2009;77:1553–60.
96. Zheng J, Meng J, Zhao S, et al. Campylobacter-induced interleukin-8 secretion in polarized human intestinal epithelial cells requires Campylobacter-secreted cytolethal distending toxin- and Toll-like receptor-mediated activation of NF-kappaB. Infect Immun. 2008;76:4498–508.
97. Ketley JM. Pathogenesis of enteric infection by Campylobacter. Microbiology. 1997;143(Pt 1):5–21.
98. Edwards LA, Nistala K, Mills DC, et al. Delineation of the innate and adaptive T-cell immune outcome in the human host in response to Campylobacter jejuni infection. PLoS One. 2010;5, e15398.
99. Monaghan T, Boswell T, Mahida YR. Recent advances in Clostridium difficile-associated disease. Gut. 2008;57:850–60.
100. Monaghan T, Cockayne A, Mahida YR. Pathogenesis of C. difficile infection and its potential role in inflammatory bowel disease. Inflamm Bowel Dis. 2015;21(8):1957–66.
101. Voth DE, Ballard JD. Clostridium difficile toxins: mechanism of action and role in disease. Clin Microbiol Rev. 2005;18:247–63.
102. Xu H, Yang J, Gao W, et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature. 2014;513:237–41.
103. Mowat C, Cole A, Windsor A, et al. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011;60:571–607.

104. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474:307–17.
105. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361:2066–78.
106. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491:119–24.
107. Lobaton T, Vermeire S, Van Assche G, et al. Review article: anti-adhesion therapies for inflammatory bowel disease. Aliment Pharmacol Ther. 2014;39:579–94.
108. Hawkey CJ. Stem cells as treatment in inflammatory bowel disease. Dig Dis. 2012;30 Suppl 3:134–9.

Chapter 2
The Enteric Nervous System

Michael Constantinescu

Abstract The gastrointestinal motility, exocrine secretions, and endocrine cells are controlled by an integrative nervous system, under the central command of the central nervous system. The enteric nervous system is considered to be quasi-autonomous and in certain circumstances may be self-sustained. The connections of the enteric nervous system with the central nervous system are through afferent and efferent neurons of the parasympathetic and sympathetic nerves, the two major pathways of the autonomous nervous system. The enteric neurons function to control the tonus of the smooth muscle in the intestinal wall and the vascular muscle motor activity and the secretory function of the gastric and intestinal glands and endocrine products and carry sensory information to the central nervous system and some function as communicators between the neurons of the intestinal wall (interneurons). Disorders of the enteric neurons may comprise dysfunctions of the secretory, motor, or immunologic functions. In this chapter, we briefly discuss some more common motility disorders.

Keywords Parasympathetic • Sympathetic • Enteric nervous system • Myenteric plexus • Submucosal plexus • Interstitial cell of Cajal • Neuroendocrine cells • Motility

The main role of the gastrointestinal system is the digestion and absorption of the food. The food that we enjoy through our senses is softened, processed by the enzymes secreted by salivary glands, and mixed with gastric, intestinal, biliary, and/or pancreatic juices and enzymes to achieve the end result of transporting the nutrients through the mucosal lining followed by the expelling of the unabsorbable

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matter by defecation. The passage of food is performed under the nervous system and endocrine control of the gastrointestinal system and its regulation of the contraction and relaxation of the digestive system muscle layers and/or sphincters. Within the peripheral nervous system, the nervous system of the gut is considered the most complex.

There are two major components: the extrinsic system that in turn is divided into two components, *the sympathetic* and *the parasympathetic* nerves, and the intrinsic system also known as enteric nervous system, considered a remote portion of the central nervous system that communicates with the central nervous system via sympathetic and parasympathetic extrinsic component neurons. The central nervous system interaction with the enteric neurons is known as "central autonomic neural network" [1]. The major physiologic processes regulated by the intricate nervous system of the gastrointestinal system include (a) the smooth muscle necessary to control motility and sphincters, (b) the mucosal role in secretion of juices and fluid-electrolyte homeostasis, (c) the cells participating in mucosal immunity, and (d) the vascular network [2].

Central Autonomic Neural Network

The enteric nervous system can function autonomously. However, the central nervous system has a major role in the control of the enteric nervous system and its functional role. There are interconnections that bring in concert the motor and sensory pathways in the central nervous system and the enteric nervous system [1, 3].

There are two major pathways of such interactions. One is the parasympathetic or craniosacral pathway. The other is the sympathetic or thoracolumbar pathway. The parasympathetic pathway has a vagal component and a sacral or pelvic component. The vagal component, the predominant participant in the parasympathetic pathway, consists of preganglionic neuron bodies in the nucleus ambiguous and the dorsal vagal nucleus in the medulla. The preganglionic neurons are cholinergic and generally have excitatory effect, increasing the gastrointestinal tract motility. The sympathetic pathway or the thoracolumbar pathway has an inhibitory role, decreasing the motility. The sympathetic system is adrenergic and consists of postganglionic fibers that innervate the gut and have the neuronal bodies in the prevertebral ganglia [1].

The innervation of the *esophagus* is supplied by the vagus nerve (predominantly parasympathetic) and sympathetic nerves that, through afferent and efferent fibers, control the muscular layer of the esophagus, its glands, and its blood vessels. The vagus nerve receives some filaments from the paravertebral sympathetic system, and, as such, it contains mixed parasympathetic and sympathetic fibers. The right vagus participates, along with the left vagus, in forming the esophageal plexus. Thereafter, the right vagus reforms as the posterior vagal trunk just before passing through the diaphragmatic esophageal hiatus. The left vagus reforms also just above

the diaphragm as the anterior vagal trunk. The surgical procedure of vagotomy was in the past more extensively used as therapeutic option for duodenal ulcer, but now is employed only occasionally. However, it is important to be aware that there are anatomic variations in the esophageal plexus and anterior and posterior trunks [4]. The esophageal sympathetic innervation is comprised of fibers with origin in the cervical and paravertebral chains. The upper portion of the esophagus is supplied by filaments from the pharyngeal plexus, and lower portions are supplied by branches from superior, middle, and vertebral ganglia of the sympathetic trunk. Esophageal filaments are also supplied by the stellate ganglia and the splanchnic nerves.

The innervation of the *stomach and duodenum* consists also of parasympathetic and sympathetic efferents and afferents. Their sympathetic nerve supply follows the gastric artery and the gastroepiploic artery and is derived predominantly from the celiac plexus. This plexus has a right and left portion, each with a celiac ganglion, with the aorticorenal ganglion and a single, unpaired superior mesenteric ganglion. The superior, middle, and inferior thoracic splanchnic nerves along with parasympathetic fibers from the posterior and anterior vagal trunk contribute to the interconnections of the celiac plexus. The celiac plexus forms nerves and networks of nerves including the left gastric plexus, right gastric plexus, hepatic plexus, superior mesenteric plexus, and splenic plexus. The phrenic plexus provides filaments to the cardia [4]. The stomach and the duodenum parasympathetic supply is provided by the anterior vagal trunk (e.g., greater anterior gastric nerve branches and pyloric branches) and by the posterior vagal trunk (e.g., greater posterior gastric nerve).

The innervation of the *small and large intestine* consists as well of sympathetic and parasympathetic nerve fibers. The sympathetic pathway has cell bodies in the prevertebral ganglia (nodose ganglia). The postganglionic fibers are reaching the small or large intestine wall through the celiac ganglia, superior mesenteric ganglion, inferior mesenteric ganglion, and superior and inferior hypogastric plexi branches and their interconnecting nerves. The parasympathetic innervation of the intestines is through the posterior vagal trunk that relays to the celiac plexus. The descending colon, the sigmoid colon, and the rectum have parasympathetic supply from the second, third, and fourth sacral spinal cord segments through the pelvic splanchnic nerves and the inferior hypogastric plexus. While the rectum is supplied from this plexus, the anal sphincter and the perianal area have direct somatic efferents and afferents from the central nervous system via the pudendal nerves [5].

Enteric Nervous System (Intrinsic System)

At the beginning of the last century, Bayliss and Starling, Magnus and Langley, and Trendelenburg found that extrinsically denervated intestines had coordinated reflex contractions (peristalsis) that were taking place by the nerves present in the intestinal wall and that the intramural intestinal neurons did not communicate with the parasympathetic neurons from the central nervous system. Millions of neurons are

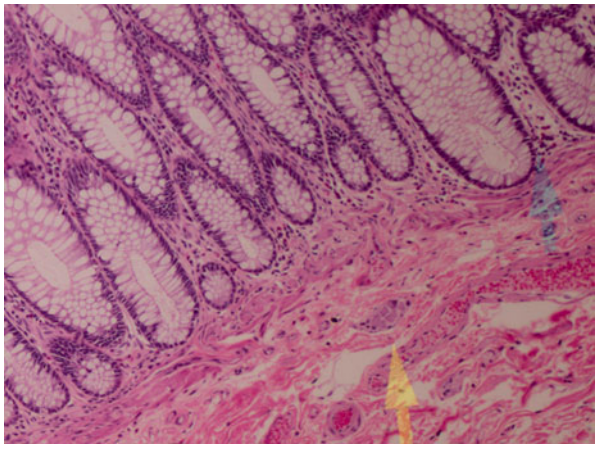

Fig. 2.1 Gastrointestinal submucosal plexus (Meissner) (*arrows*) composed of ganglion cells and Schwann cells, hematoxylin and eosin stain, magnification 10×

identified in the gastrointestinal system. The functional and chemical makeup of the intestine showed that, like the central nervous system, the enteric nervous system contains sensory and motor neurons and interneurons and that the synaptic chemical connections direct the integrative network from sensory to interneurons to motor neurons and to effector system in a similar manner to the central nervous system [6].

In the intestinal wall, there are small ganglia composed of groups of nerve cell bodies. Nerve processes from these small ganglia form three main intramural plexi (Figs. 2.1, 2.2, 2.3 and 2.4). The submucosal plexus is named Meissner plexus. The deep submucosal plexus is known as Henle's plexus (1871). The intermuscular, or myenteric plexus is also known as Auerbach plexus. It is the most easily seen histologically and is found throughout the gastrointestinal system between the circular smooth muscle layer of the muscularis propria and the longitudinal muscle layer. It also innervates the motor end plates of the striatal muscle portion of the esophagus with the release of the nitric oxide [1]. Its main role is to provide innervation to the two muscle layers in the muscularis propria, and it has an additional role in the innervation of the mucosa. Immediately beneath the muscularis mucosa is the Meissner plexus, the submucosal plexus. It is composed of neurons or ganglion cells and glial cells (Schwann cells). These are interspersed among the loose stromal elements of the submucosa [7]. Occasionally, ganglion cells may be seen in the lamina propria of the mucosa, but it is abnormal to find an increased number of ganglion cells here or clustered ganglion cells in the lamina propria. In such cases, conditions such as ganglioneuroma, inflammatory bowel disease, or neurofibromatosis should be considered. The deep submucosal plexus of Henle is located along the inner portion of the muscularis mucosa. The deep submucosal plexus has fewer small neurons compared to the Meissner plexus. In the latter, approximately 50 % of the ganglia were associated with single fiber tracts, as compared to the deep submucosal plexus (Henle's) in which approximately 75 % were associated with single fiber tracts [8].

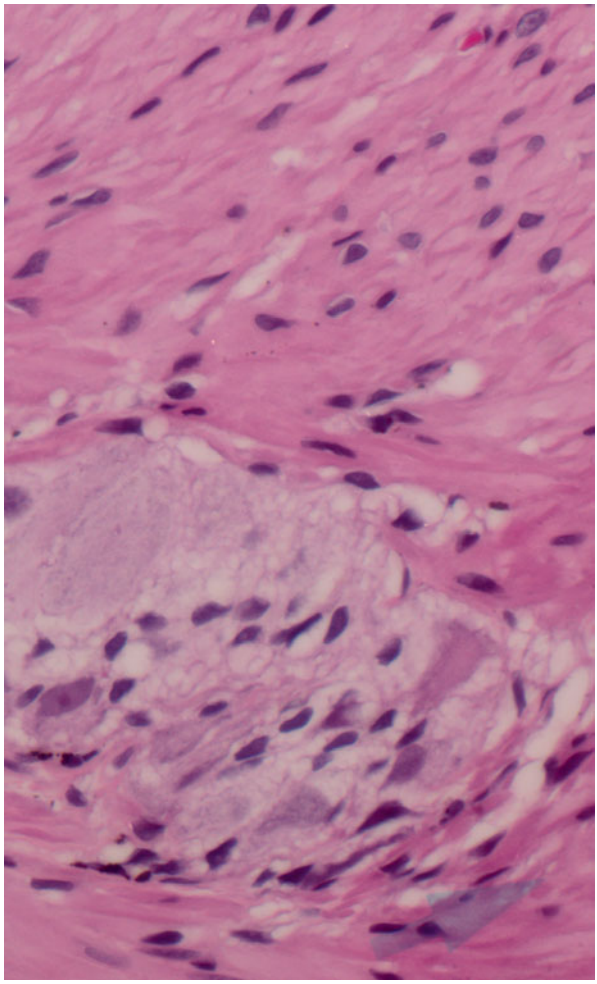

Fig. 2.2 Gastrointestinal myenteric plexus (Auerbach) (*arrow*), composed of nerve fibers, ganglion cells, and Schwann cells, hematoxylin and eosin stain, magnification 40×

The Enteric Neurons

The enteric neurons have been classified in primary afferent neurons, excitatory circular muscle motor neurons, inhibitory circular muscle motor neurons, longitudinal muscle motor neurons, ascending interneurons, descending interneurons, secretomotor and vasomotor neurons, and intestinofugal neurons [3].

Primary afferent neurons or the intrinsic primary afferent neurons may be seen in ganglia of both the Meissner and the Auerbach plexi. Approximately 30 % of myenteric neurons and 14 % of submucosal ganglia neurons are primary afferent neurons. These neurons respond to chemical or mechanical stimuli of the mucosa to muscle tension and radial stretch of the intestinal wall [3].

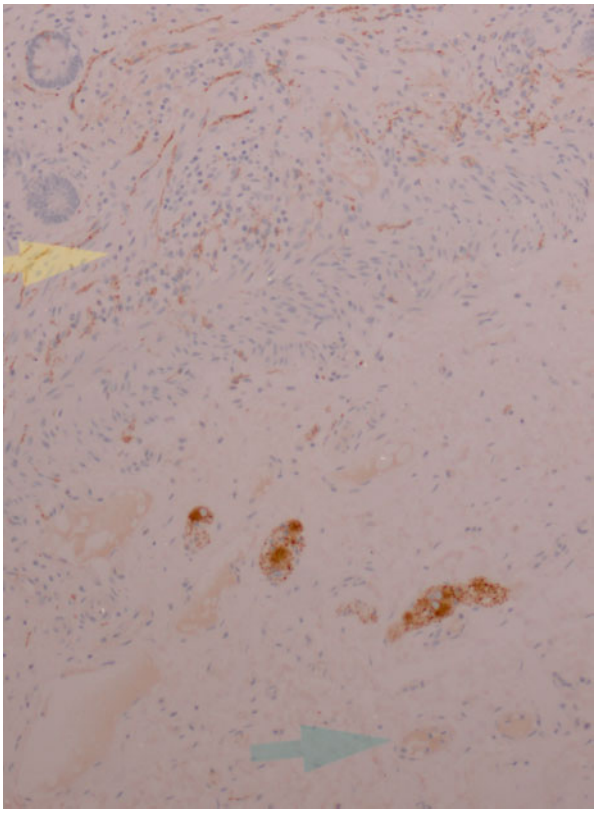

Fig. 2.3 Gastrointestinal submucosal plexus (Meissner) (*arrows*), its ganglion cells underlined by immunohistochemistry stain with synaptophysin, magnification 10×

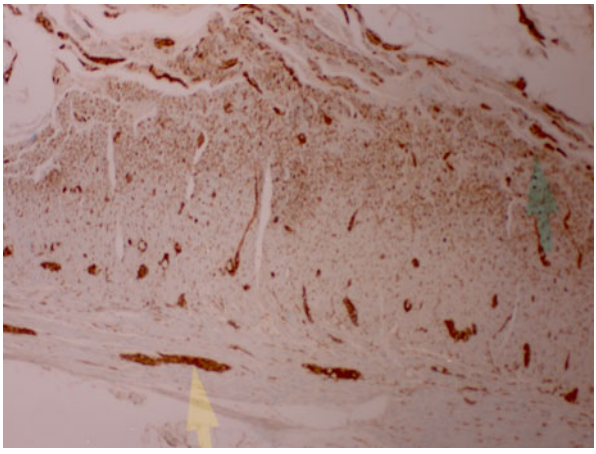

Fig. 2.4 Gastrointestinal submucosal and myenteric plexi (*arrows*) as underlined by the neural immunohistochemistry marker S100, magnification 4×

The excitatory circular muscle motor neurons are the final effector in the circular layer of the muscularis propria. They are considered to receive fast nicotinic and slow synaptic input from the intrinsic primary afferent neurons. In the deep myenteric plexus, they are denser, and, via acetylcholine and tachykinin transmitters, they act predominantly directly on the circular smooth muscle.

Inhibitory circular muscle motor neurons also receive fast nicotinic input from the intrinsic primary efferent neurons. They also receive noncholinergic input. They act directly and indirectly, by the production of nitric oxide, adenosine triphosphate, vasoactive intestinal peptide (VIP), and other peptides, on the circular smooth muscle, having an inhibitory effect.

The longitudinal muscle motor neurons receive their input from the intrinsic primary afferent neurons and from interneurons.

Ascending interneurons are forming chains of excitatory interneurons with other interneurons and receive fast nicotinic synaptic input and slow synaptic input from the intrinsic primary afferent neurons. They are participating in the action of the excitatory circular muscle neurons. These interneurons contain, along with the enzymes needed for acetylcholine synthesis, opioid peptides and tachykinins.

The descending interneurons are more likely cholinergic. There are some containing choline acetyltransferase and some containing somatostatin as neurotransmitters. Some contain also serotonin. They receive input from non-primary afferent neurons and make synapses with submucosal and myenteric neurons.

Some of the secretomotor neurons are cholinergic, while some contain VIP. They project to the mucosa, to the myenteric plexus ganglia, and to the submucosal plexus ganglia. The secretomotor neurons that contain VIP have inhibitory synapses from the extrinsic sympathetic pathway and possibly from other myenteric neurons. Some submucosal neurons are cholinergic and project to the small blood vessels.

The intestinofugal neurons are cholinergic neurons that project to the prevertebral ganglia from myenteric plexus ganglia [3].

Interstitial Cells of Cajal

In the gastrointestinal motility, besides the extrinsic and intrinsic nerve supply, other components also participate. The interstitial cells of Cajal (ICC) are an important part of the gastrointestinal neuromuscular function. When Cajal described these cells in 1893, he identified them as "primitive neurons." Electron microscopy showed that these cells are either fibroblast-like cells or primitive muscle cells. These cells express c-kit (CD117), a tyrosine kinase receptor. It was shown that these cells are pacemaker cells for the gastrointestinal tract. They are organized in a network that propagates slow wave electrical activity. The slow waves propagate from ICC to the smooth muscle fibers. Slow waves produce depolarization, followed by the entrance of calcium into the muscle cells, resulting in phasic contraction, or peristalsis. Another role of ICC is in the mediation of the input from the enteric motor neurons to the smooth muscle. ICC also provides sensitivity to stretch. Most ICCs are located at the periphery of myenteric plexus (Auerbach plexus). They form a network from the branched processes of these multipolar ICC. Other ICCs are located within the circular or longitudinal smooth muscle layers, in the connective tissue, within the submucosal plexus (Meissner plexus), or within the deep muscular plexus [9]. The gastrointestinal stromal tumors (GISTs) are mesenchymal tumors of the gastrointestinal system that express c-kit (CD117), CD34, and

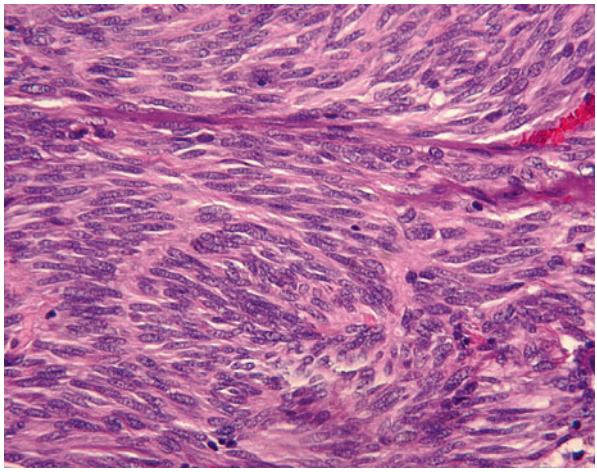

Fig. 2.5 Gastrointestinal stromal tumor of the stomach, a proliferation of interstitial cells of Cajal, hematoxylin and eosin stain, magnification 40×

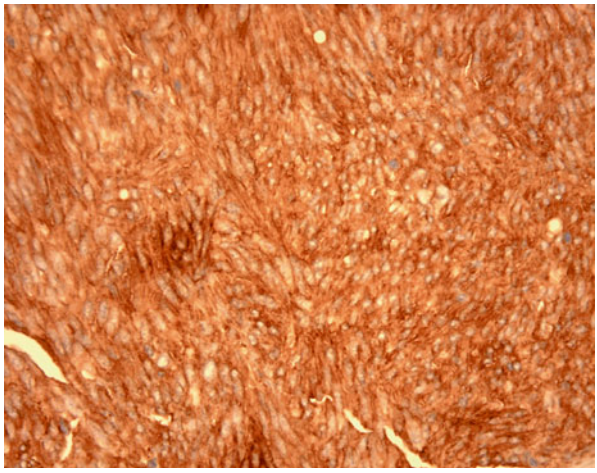

Fig. 2.6 Gastrointestinal stromal tumor of the stomach; the tumor proliferation of interstitial cells of Cajal is demonstrated by the expression of CD117(c-kit) marker (immunohistochemistry, magnification 40×)

DOG-1. In 1984, Herrera described such tumors as "plexosarcoma," indicating that these tumors are originating in the enteric plexus [10]. Today it is widely accepted that GIST is derived from ICC (Figs. 2.5, and 2.6). In addition to the surgical treatment, the current therapy for GIST includes a c-kit tyrosine kinase inhibitor, imatinib, with a good response [11].

Neurotransmitters of Enteric Motor Neurons

As we have seen, the motor neurons may be excitatory or inhibitory. The neurotransmitters released by excitatory neurons are necessary for contraction and mucosal gland secretion. Their main neurotransmitters are acetylcholine and substance

P. Enteric gland secretions are stimulated by excitatory neurotransmitters acetylcholine, ATP, and the vasoactive intestinal peptide (VIP).

The inhibitory neurotransmitters for inhibitory neurons that suppress the smooth muscle contraction are ATP, VIP, and nitric oxide.

Sensory Information (Extrinsic Afferent Supply) of the Gastrointestinal Tract

As previously noted, there is a rich afferent innervation that carries the sensory information from the gut to the central nervous system and mediates the sensations from the gastrointestinal organs. The afferents are carried by the vagal and splanchnic and pelvic nerves. The vagus nerves have the cell bodies in the nodose ganglia and then end centrally in the nucleus of the tractus solitarius. The splanchnic afferent neurons have their cell bodies in the dorsal root ganglia. The vagal afferents are predominantly in the upper part of the gastrointestinal system, while the pelvic afferents supply mainly the lower portion of the large intestine. The entire gastrointestinal system appears to send extrinsic afferent information through the splanchnic nerves [12]. The vagal afferent neurons are detecting mechanical distension and are sensitive to the glucose, amino acid, or long-chain fatty acid concentrations in the intestinal lumen. The mucosal neuroendocrine cells release chemical transmitters necessary for the vagal afferent activity. An example is the increased release of serotonin by the enterochromaffin endocrine cells, due to mucosal damage during chemotherapy, with consequently severe emesis. Increased serotonin acts on 5-hydroxytryptamine-3 receptors (5-HT_3) located on the vagal neurons. The primary vagal afferent neurons, in turn, are connected with the neurons in the brain stem that control vomiting. The antiemetic drug ondansetron is a 5-HT_3 receptor antagonist; therefore, it inhibits the vagal primary afferent neurons. The splanchnic primary afferent neurons are sensing pain (nociceptive receptors). They are activated by chemical, mechanical, or thermal stimuli. Some of their neurotransmitters include calcitonin gene-related peptide and substance P.

In the myenteric plexus, some neurons contain opioid type of peptides (e.g., enkephalins, dynorphin, and endorphin). The opioid receptors are localized principally to the enteric neurons, and opioid receptor agonists produce a decreased neuronal excitability, resulting in motor inhibition and constipation [13].

Neuroendocrine Cells or Endocrine Cells?

The neuroendocrine cells in the gastrointestinal system have important functions. Their embryological derivation has been controversial. These cells have endocrine and paracrine, and some have neurotransmitter functions. Many of these cells have distinctive neural markers. When immunohistochemistry stains are performed,

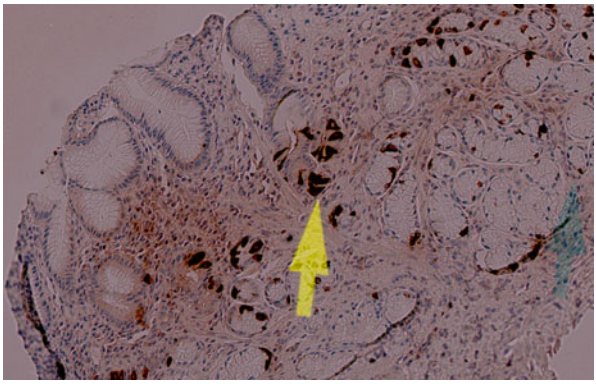

Fig. 2.7 Neuroendocrine cells in the gastrointestinal tract. The enterochromaffin-like cells (*arrows*) associated with the gastric glands can be easily visualized by immunohistochemistry stains for synaptophysin. Magnification 10×

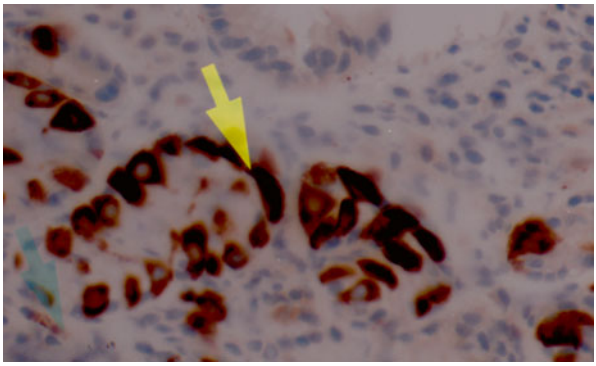

Fig. 2.8 Neuroendocrine cells. Enterochromaffin-like cells (ECL) hyperplasia (*arrow*) in the stomach, an increased number of ECL, as underlined by synaptophysin immunohistochemistry stain, magnification 40×

they express either chromogranin or synaptophysin or both. Synaptophysin stains synaptic neural granules and chromogranin granules that are found in neurons or endocrine cells (granins). However, while some neuroendocrine cells (e.g., those in the thyroid) have more neural-like features (neural crest rather than endodermal origin), those located in the gastrointestinal tract display more epithelial-like properties (endodermal origin rather than neural crest) [14]. In the gastrointestinal tract, they have wide distribution in the distal esophagus, stomach, small intestine, large intestine, and anus. Many are in the epithelium or lamina propria of the mucosal lining. They have sensitivity for thermal, chemical, and mechanical stimuli. Some of their products are true peptide hormones with release through the bloodstream to reach their targeted tissue into the circulation (e.g.. gastrin, secretin). Other paracrine cells have only local effect on the nearby cells (e.g., somatostatin). There are known interactions between the endocrine cells and enteric neurons, between neurons and endocrine cells, and among various endocrine cells [2]. The neuroendocrine proliferation in the gastrointestinal tract results in neuroendocrine tumors. In the stomach, there are early precursors noted, enterochromaffin-like cell (ECL) proliferation. These occur predominantly in the context of atrophic gastritis as ECL hyperplasia that may progress to ECL dysplasia and then to well-differentiated neuroendocrine tumors (carcinoid) [15] (Figs. 2.7, 2.8, 2.9, 2.10, and 2.11).

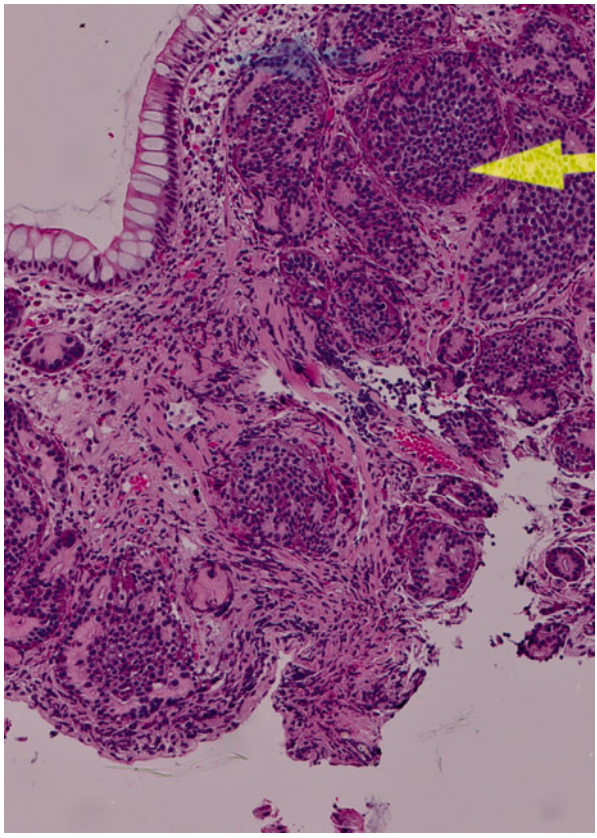

Fig. 2.9 Neuroendocrine cell tumor proliferation, low-grade neuroendocrine tumor (carcinoid) (*arrow*) in the small intestine, hematoxylin and eosin stain, magnification 10×

Selected Disorders of the Nervous System of the Gut

Gastrointestinal Neuromuscular Disorders

This is a very heterogeneous group of conditions that have as common denominator alterations in the motility function of the gastrointestinal tract resulting in inadequate propulsion and emptying functions.

Achalasia

In the lower esophagus, nonselective loss of all the enteric neurons from the Auerbach's plexus (myenteric plexus) results in *achalasia*, a condition characterized by unrelaxed lower esophagus sphincter, with a persistent tonic contraction. As a consequence, there is a functional esophageal obstruction (cardiospasm), with

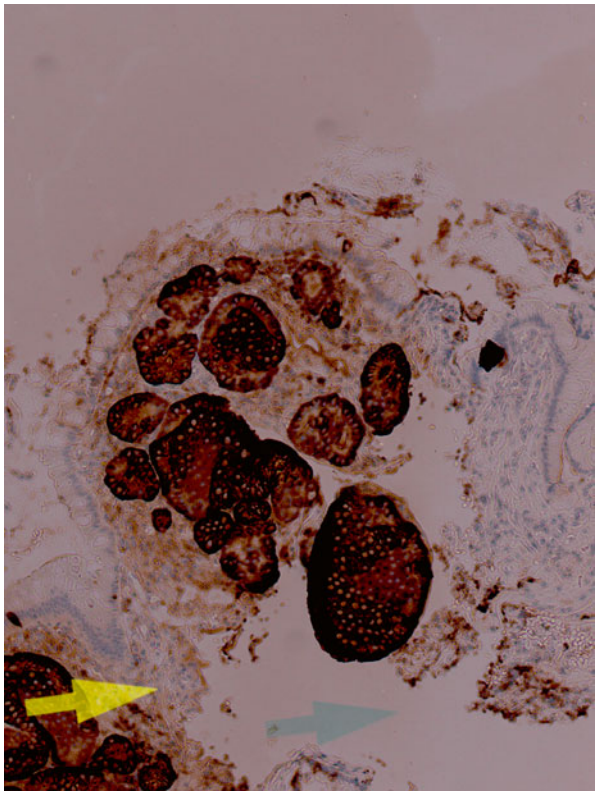

Fig. 2.10 Neuroendocrine tumor, carcinoid (*arrow*), as evidenced by the immunohistochemistry stain marker, synaptophysin, magnification 10×

esophageal dilation and hypertrophy. Achalasia may also occur when there is selectively a dysfunction or loss of the inhibitory myenteric plexus neurons that contain VIP or nitric oxide. Histologically, there is significant decrease or absence and/or degeneration of the myenteric plexus neurons [16].

Pyloric Stenosis

Pyloric stenosis is a rather common condition in infants (1/3000) that has a high familial predilection and is more common in males. Its etiology is disputed, but one theory is the delay in the innervation of the pyloric region and decreased or absent nerves or neurotransmitters such as nitric oxide, absence of ICC, and other supporting cells. The pyloric muscle is hypertrophic. Histologically, there is degeneration of glial cells, increased number of Schwann cells, and hypertrophy of the nerves. ICCs are absent or significantly decreased. ICCs contain the inhibitory neurotransmitters nitric oxide [16].

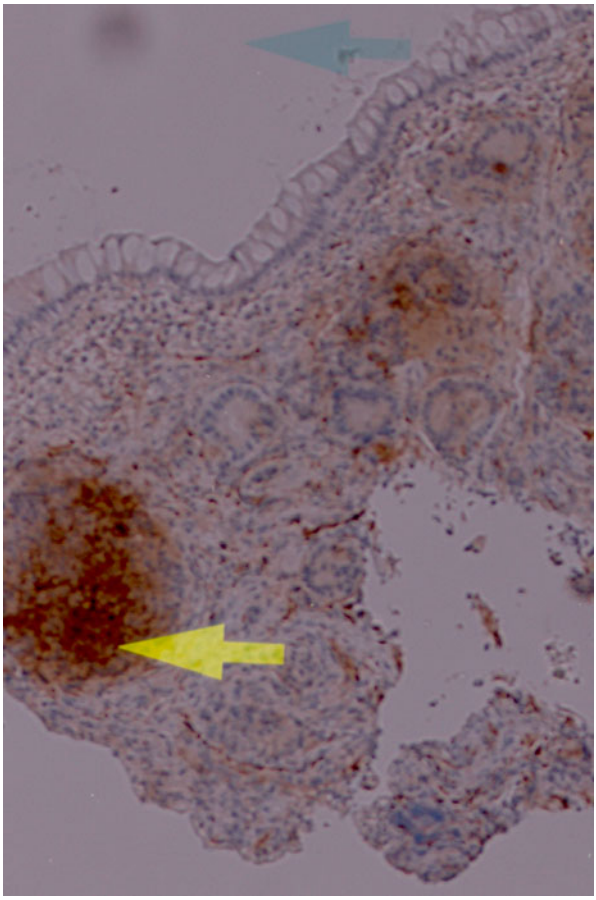

Fig. 2.11 Neuroendocrine tumor, carcinoid (*arrow*), as evidenced by the immunohistochemistry stain marker, chromogranin, magnification 10×

Intestinal Pseudo-obstruction

Intestinal pseudo-obstruction is a group of acute or chronic disorders that in the absence of mechanical obstructions show an absence of the propulsive function of the intestine. The chronic pseudo-obstruction may be idiopathic, of unknown pathogenesis. There are several rare diseases included under this classification. They include conditions such as idiopathic megacolon or megaduodenum. As a prototype we will use Hirschsprung's disease. It is by definition a disease of newborn or infant involving the sigmoid and rectum, predominantly in males. Characteristically a segment of distal colon or rectum lacks ganglion cells, especially the ganglion cells of the Meissner (submucosal) plexus. This segment is aperistaltic and narrow. Proximal to it, the colon dilates (congenital megacolon) [16].

Chronic or acute pseudo-obstructions may be also secondary to systemic diseases: endocrine disease such as hypothyroidism; systemic disorders involving the

smooth muscle such as amyloidosis or myotonic dystrophies; systemic conditions involving the extrinsic nervous system such as stroke, diabetes, or orthostatic hypotension; or systemic intrinsic enteric nervous system disorders such as paraneoplastic syndrome, viruses, drugs, or Chagas' disease. This latter group of conditions is generally characterized by multiple segments of the gastrointestinal tract with loss of ganglion cells [1, 16].

Neuronal Dysplasia (Ganglioneuromatosis)

Ganglioneuromatosis may be either nodular or diffuse and consists of a proliferation of ganglion cells, nerve fibers, and Schwann cells, either at myenteric plexus level or throughout the thickness of the intestinal wall (Figs. 2.12 and 2.13). It may

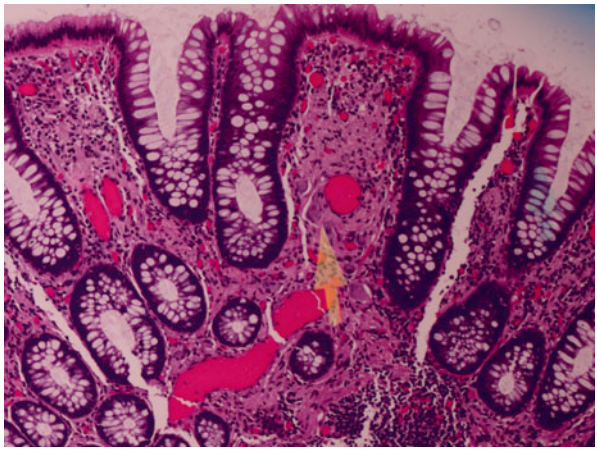

Fig. 2.12 Ganglioneuroma, a proliferation of ganglion cells (*arrow*) in the submucosal plexus of Meissner, with cells abnormally seen in the mucosal lamina propria of the colon. Hematoxylin and eosin, magnification 10×

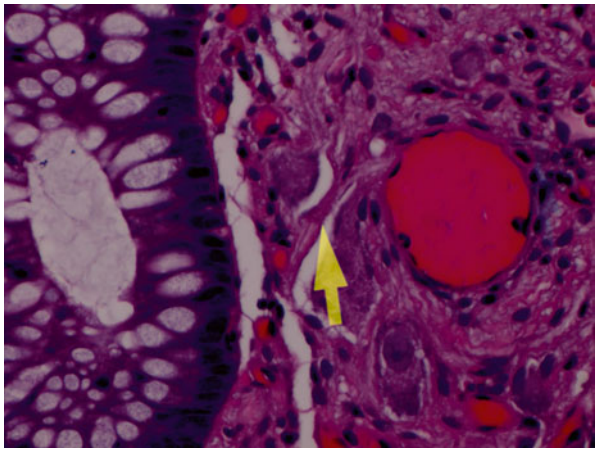

Fig. 2.13 Ganglioneuroma, a proliferation of ganglion cells (*arrow*) in the submucosal plexus of Meissner, with cells abnormally seen in the mucosal lamina propria of the colon. Hematoxylin and eosin, magnification 40×

be a cause of pseudo-obstruction. In some patients, this condition has been associated with multiple endocrine neoplasia type 2B (MEN-2B) [1].

Other Entities

Other enteric neuron disorders such as neurogenic secretory diarrhea and neurogenic constipation, abdominal pain, inflammatory bowel disease, irritable bowel syndrome, and neuroimmunological modulation are discussed elsewhere in this book.

Conclusion

This chapter has briefly demonstrated that gastrointestinal function is rigorously controlled and integrated with the central nervous system. The enteric neurons that participate in motility, the neurons that help regulate the glandular and endocrine secretions, the vasomotor neurons, the afferent sensory neurons bringing information from the gut, and the interneurons and transmitters with role to facilitate the communication between neurons are working in concert for the complex function of the gastrointestinal system.

References

1. Goyal RK, Hirano I. The enteric nervous system. N Engl J Med. 1996;334:1106–15.
2. Noffsinger A, Fenoglio-Preiser C, Maru D, Gilinsky N. Atlas of nontumor pathology: gastrointestinal disease. Washington, DC: American Registry of Pathology and Armed Forces Institute of Pathology; 2007.
3. Costa M, Brookes SJ, Hennig GW. Anatomy and physiology of the enteric nervous system. Gut. 2000;47(S 4):iv15–9.
4. Netter FH. Digestive system. Upper digestive system (part 1). Summit, ng: The CIBA Collection of Medical Illustrations; 1959.
5. Netter FH. Digestive system. Lower digestive tract (part 2). Summit, ng: The CIBA Collection of Medical Illustrations; 1962.
6. Wood JD. Neuropathophysiology of functional gastrointestinal disorders. World J Gastroenterol. 2007;13:1313–32.
7. Sternberg SS, editor. Histology for pathologists. Philadelphia/New York: Lippincott Raven; 1997.
8. Hoyle CH, Burnstock G. Neuronal populations in the submucous plexus of the human colon. J Anat. 1989;166:7–22.
9. Al-Shboul OA. The importance of interstitial cells of Cajal in the gastrointestinal tract. Saudi J Gastroenterol. 2013;19:3–15.
10. Herrera GA, de Pinto MH, Grizle WE, et al. Malignant small bowel neoplasm of enteric plexus derivation (plexosarcoma): light and electronic microscopic study confirming the origin of the neoplasm. Dig Dis Sci. 1984;29:275–84.
11. Streutker CJ, Hulzinga JD, Driman DK, et al. Interstitial cells of Cajal in health and disease: part II, ICC and gastrointestinal stromal tumours. Histopathology. 2007;50:190–202.

12. Berthoud HR, Blackshaw LA, Brookes SJ, et al. Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract. Neurogastroenterol Motil. 2004;16(S1):28–33.
13. Galligan JJ, Akbarali HI. Molecular physiology of enteric opioid receptors. Am J Gastroenterol Suppl. 2014;2:17–21.
14. Rosai J. The origin of neuroendocrine tumors and the neural crest saga. Mod Pathol. 2011;24:S53–7.
15. Hamilton SR, Aaltonen LA, editors. Pathology and genetics. Tumors of the digestive system. World Health Organization classification of tumors. Lyon: IARC Press; 2000.
16. Riddell R, El-Zimaity H, Jain D. Motility disorders, in Lewin, Weinstein and Riddell's gatrointestinal pathology and its clinical implications, vol. I. Philadelphia: Wolters Kluwer Health; 2014. p. 226–89.

Chapter 3
Microbial Regulation of Gastrointestinal Immunity in Health and Disease

Sheila Patrick, Rebecca J. Ingram, Thamarai Schneiders, and Denise C. Fitzgerald

Abstract The gastrointestinal (GI) tract represents the front line of microbial-host interaction by virtue of its immense surface area and constant microbial supply from ingested food. The gastrointestinal immune system shapes the communities of microbes throughout the GI tract, and in turn, the microbiota provide metabolites and other cues to support the development and normal function of the immune system. Emerging research shows that this influence on the immune system encompasses both innate and adaptive immunity and extends beyond the gut to anatomical sites throughout the body. This chapter presents an overview of the microbiology and immunology of the GI tract, examines microbial population dynamics revealed by studies such as the Human Microbiome Project and discusses the potential impact of emerging antimicrobial resistance to the microbiota and human health.

Keywords Microbiota • Immunity • Gut-associated lymphoid tissue (GALT) • *Bacteroides* • Metabolites • Inflammation • Antimicrobial resistance

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Introduction

Within the gut resides an extensive commensal microbial community with which the host shares a symbiotic relationship that is tightly regulated by the immune system. Understanding both the microbiology and the immunology of the gut reveals a complex and dynamic environment that is characterised by specific anatomical and functional niches, complex regulatory mechanisms and symbiotic metabolism. In this chapter, we will discuss both microbial distribution and function and immunological characteristics of the gut and address how the emergence of antimicrobial resistance may alter the gut microbiome.

Regular breaches of the gut epithelial barrier expose the submucosa to commensal bacterial antigens; however, a combination of innate and adaptive immune responses rapidly reinforces barrier function and limits inflammatory response during such routine exposures. Most organisms that are able to breach the physical barrier of the gut are rapidly phagocytosed by macrophages located in the subepithelium. Whilst intestinal macrophages are particularly adept at bacterial clearance, these cells express little inflammatory cytokine and therefore rarely trigger overt inflammation following bacterial uptake. Presumably, this is an adaptive mechanism to prevent constant activation of inflammation by the gut commensals and food antigens. This is in part mediated by the fact that very few intestinal macrophages express TREM-1, which would normally help drive the amplification of the inflammatory response [54].

However, it is not just friendly neighbourhood commensal bacteria that the gastrointestinal mucosa is exposed to; the tissue must also respond to highly pathogenic organisms. The gastrointestinal tract is susceptible to infection by bacterial, viral and parasitic organisms. For infection to occur, there must be sufficient microbes that possess sufficient virulence factors and capacity to overcome or circumvent the host immune response. Tissue tropism describes infection of a specific organ in the host, and a number of pathogens demonstrate specific tropism for the gut (e.g. *Giardia*, *Cryptosporidium*). Other infections initiate in the gut but may not be confined to this site and have the potential to become systemic infections (e.g. *Salmonella*, *Enterovirus*). Much of microbial population dynamics are regulated not only by the host immune system but by the competitive microbial environment of the gut.

Overview of Microbiology of the Gastrointestinal (GI) Tract

The normal resident microbiota is an integral part of the mammalian host, inextricably linked with the normal development and functioning of the eukaryotic component. This is exemplified by a growing understanding of the role of the GI tract microbiota in immunity and its impact on health and disease.

The gastrointestinal tract is an effective open-ended culture system for microbes, with faeces as the product. To the human host, however, faeces are not the only

outcome of microbial colonisation. De-conjugation of bile salts and subsequent recycling via the liver, the degradation of ingested plant polysaccharides to short chains fatty acids utilised by the intestinal mucosal epithelium and the provision of vitamins are all associated with the resident microbiota [11]. It has also become increasingly clear that microbial activity is key to immune system (IS) development. Our understanding of the intimate microbiota/immune system relationship has progressed from a knowledge that immune system development does not reach its full repertoire in 'germ'-free animals [20] to the beginnings of a better understanding of key molecular relationships in health and disease. This progress has been informed by an increased understanding of microbiota complexity, underpinned by complete genome sequencing of individual species (e.g. [8, 47]) metataxonomics based primarily on 16S sequencing and metagenomics based on whole community shot-gun sequencing (e.g. Human Microbiome Project Consortium 2012, [51]). It should be noted that sampling protocols, DNA extraction methods and DNA storage conditions prior to sequencing can have a significant impact on the detected abundance of different bacterial taxa. For example, some methodologies potentially underestimate the *Bacteroidetes* content [62]. This may impact on the interpretation of much of the already published data and the comparison of different studies in relation to relative abundance of different groups of microbes.

The normal resident microbiota of the human gastrointestinal tract includes bacteria, archaea, protozoa, yeast and associated viruses. These microbes colonise along the GI tract in varying numbers, with bacterial colonisation increasing along the GI tract, from the stomach, duodenum and jejunum (upper small intestine) with relatively fewer bacteria to greater numbers in the ileum (distal small intestine) and large numbers in the colon [11]. Estimates of microbial colonisation range from 10^2 to 10^7 per gramme in the small intestine and $>10^{11}$ per gramme in the large intestine. Despite technological advances, much remains to be understood about the complex interactions amongst this diverse community and between this community and the human host. Bacteria have been the focus of most studies, due to their predominance within the GI tract community; indeed, it has been estimated that bacteria constitute up to 30 % or more of faecal matter. The GI tract, in particular in the large intestine, is a low redox anaerobic environment. During the long evolutionary relationship and the microbial adaptation to this environment, many of the microbes have developed a strictly anaerobic metabolism. The predominant species are unable to multiply in the presence of oxygen and many are killed by exposure to oxygen. With rigorous adherence to anaerobic culture techniques, 10^{11} or more viable bacteria can be isolated per gramme of faeces (Table 3.1; [45]).

By culture, the consistently predominant bacterial genus isolated from faeces is *Bacteroides*, with variable reports of the prevalence of other bacterial genera (Table 3.1). It is also of interest that some individuals may have up to 10^{11} viable archaea of the genus *Methanobrevibacter*. Comprehensive information pertaining to earlier investigation of the normal microbiota of humans and animals by culture can be found in the following studies [13, 20, 21]. Metagenomic sequencing has largely confirmed the earlier culture data in relation to the predominant genera present within the faecal microbiota (Fig. 3.1) [3, 61]. The major phyla detected are the

Table 3.1 Major genera of bacteria and archaea in the adult human faecal microbiota determined by culture

Bacteria	Gram reaction	Morphology	Total viable count[a] (per g or faeces)
Bacteroides	–	Rod	10^9–10^{14}
Eubacterium	+	Rod	10^5–10^{13}
Bifidobacterium	+	Rod	10^5–10^{13}
Clostridium	+	Rod	10^3–10^{13}
Lactobacillus	+	Rod	10^4–10^{13}
Peptostreptococcus	+	Coccus	10^4–10^{13}
Ruminococcus	+	Coccus	10^5–10^{13}
Streptococcus	+	Coccus	10^7–10^{12}
Methanobrevibacter	+	Coccobacillus	10^7–10^{11}
Desulfovibrio	–	Rod	10^5–10^{11}
Fusobacterium	–	Rod	10^9
Enterococcus	+	Coccus	10^7
Escherichia coli	–	Rods	10^7
Prevotella/Porphyromonas	–	Rods	10^4

Adapted from Patrick [45]
[a]Compiled from Gibson [21] and Willis [65]

Firmicutes, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. The relative proportions of these phyla vary dependent on the location within the GI tract, with the *Firmicutes* and *Bacteroidetes* major groups present in the colon [60]. The *Firmicutes* are a diverse group of low %GC Gram-positive bacteria. Predominant representative genera within faeces include *Faecalibacterium*, *Roseburia* and *Eubacterium. Faecalibacterium prausnitzii* (formerly *Fusobacterium prausnitzii*: [12]) is taxonomically within the class *Clostridium* and family *Ruminococcaceae*. As a single species, it is highly prevalent in the faeces of most healthy individuals, where it may account for more than 5 % of the bacterial population. This bacterium is killed rapidly on exposure to oxygen and is termed 'extremely oxygen sensitive' [38]. As a single genus, however, *Bacteroides* spp. of the 'fragilis group' are clearly key members of the faecal microbiota as determined by both culture and non-culture methods (Table 3.1, Fig. 3.1). In contrast to *F. prausnitzii*, although *Bacteroides* require strictly anaerobic conditions for optimal isolation and growth, *Bacteroides* are relatively more aerotolerant; there is evidence that *Bacteroides* spp. can grow in the presence of nanomolar concentrations of oxygen and have been termed 'nanaerobes' [4]. *Bacteroides* spp., in particular *B. fragilis*, are also associated with potentially life-threatening infection if they have the opportunity to colonise sites other than the GI tract, for example, as a result of rupture of an inflamed appendix or GI tract surgery. Detailed review of the *Bacteroides* can be found in [46] and [63]. Clearly, the oxygen tolerance of *Bacteroides* is potentially a factor in its success as an opportunistic pathogen; whether or not the difference in oxygen tolerance between *F. prausnitzii* and *Bacteroides* relates to the roles of these bacteria in health and disease in the GI tract remains to be determined.

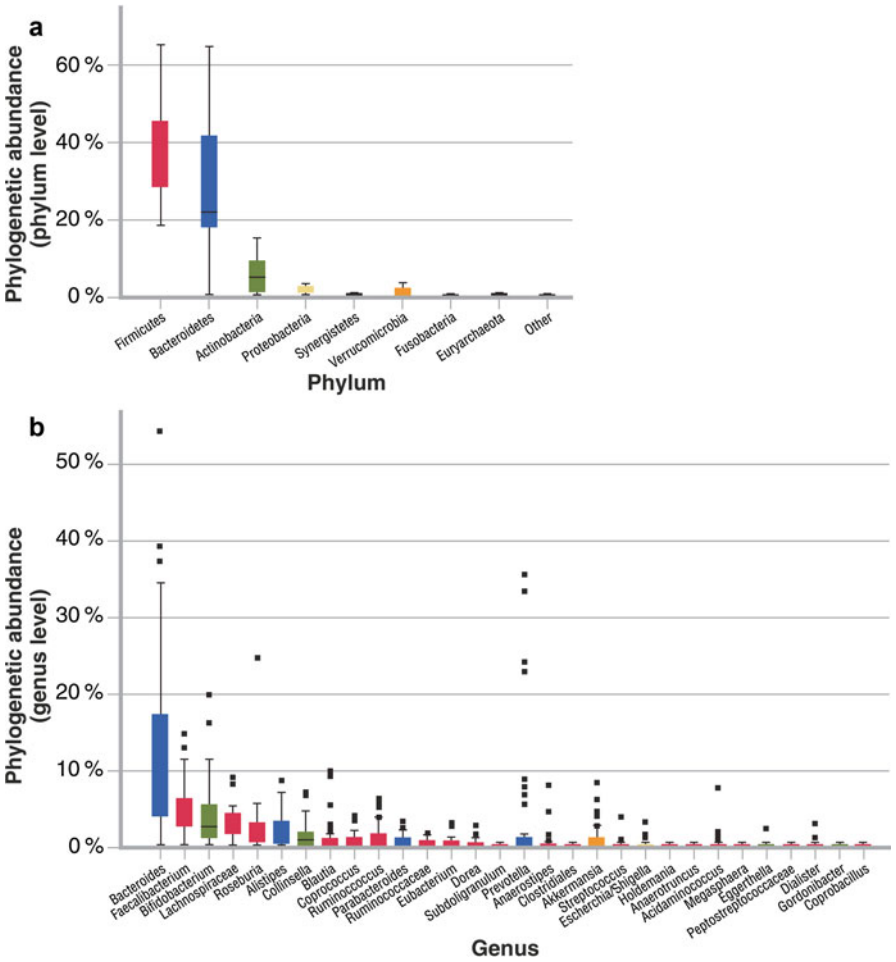

Fig. 3.1 The relative abundance of phyla (**a**) and genera (**b**) of the human gut microbiome as determined by metagenomic sequencing. Colour codes for the predominant phyla: Bacteroidetes, blue; Firmicutes, red; Actinobacteria, green; Proteobacteria, yellow; Verrucomicrobia, orange (Modified from Arumugam et al. [3])

The majority of GI tract microbiota studies have focused on faeces, likely due to the relative ease of obtaining faeces compared with mucosal biopsy in particular from healthy individuals. However, it should be noted that the faecal composition may not necessarily reflect the mucosal associated population, the primary site of key importance to immune system interactions. For example, the mucosa-adherent *Bacteroides* spp distribution may be considerably different from the faecal, with *B. fragilis* being found in greater numbers, relative to *B. vulgatus*, than in faeces [42, 50]. In addition, the faecal microbiota differs from the small intestine in relation to diversity and number of microbes. Sampling, representative of the natural

state, from the small intestine of healthy individuals is challenging; techniques used include subjects swallowing a capsule attached to a catheter for the collection and aspiration of luminal fluid. By using this technique, Henriksson and colleagues [26] determined that the predominant genera in the proximal jejunum of 11 healthy individuals consisted of predominantly *Streptococcus*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Peptostreptococcus*, *Eubacteria*, *Veillonella*, *Bacteroides* and *Fusobacterium* spp. [26]. Sampling ileostoma effluent from individuals with an abdominal stoma connected to the terminal ileum as a result of having undergone colectomy has provided some insights into the jejunal and proximal-ileum microbiota [14]. These authors reported that *Streptococcus* and *Veillonella* are consistently associated with the small intestine. The small intestine microbiota is more varied than the faecal microbiota, both between individuals and also over time, content may be more subject to fluctuation due to ingestion and may reflect the oral microbiota. Similarities with the genera present in saliva were clearly evident in the study of Henriksson et al. [26]. The intimate association of microbes with the small intestinal mucosa, in particular in the ileum, may be a key factor in immune system interactions [14].

There has been much recent focus on understanding dysbiosis at the level of bacterial populations. This is defined as a shift from a normal state of symbiosis to an increase within the resident microbial population of bacteria that induce a pro-inflammatory response, local to the GI tract, which is a potential initiator or driver of disease. The potential for intimate molecular interaction between individual microbes and the human host should not, however, be overlooked. Individual microbe/host interaction is epitomised by *B. thetaiotaomicron* influence on Paneth cells in the mouse ileum. During the differentiation of Paneth cells, alterations occur in the composition of cell surface oligosaccharides (glycoconjugates). In germ-free mouse epithelium, Paneth cell glycoconjugates become fucosylated, but when mice are raised with a conventional microbiota, not only Paneth cells but also enterocytic and goblet cell lineages became fucosylated. Colonisation of germ-free mice with a pure culture of *B. thetaiotaomicron* also restores full glycosylation, whereas an isogenic transposon insertion mutant of *B. thetaiotaomicron*, incapable of using L-fucose as a carbon source, does not. The bacterium induces fucosylation of the villus glycoconjugates, subsequently cleaves the fucose with secreted alpha-fucosidase and uses the fucose as a carbon and energy source. This effect is not observed in large intestine (caecal and colonic) epithelium of germ-free and colonised mice, which are both fucose positive [7]. As the major site of colonisation by *Bacteroides* spp. is in the large intestine rather than the small intestine, this appears to represent specific adaptation to the small intestinal environment. This exemplifies the danger that by focusing only on microbial population shifts, important individual microbe molecular interactions may be missed. In the context of GI tract/immune system interactions, individual microbe interactions at the mucosal surface are likely to also be of key importance and should be considered in parallel with microbial population dynamics.

A combination of operon divergence over evolutionary time and horizontal gene transfer has led to an extensive *B. fragilis* pan-genome which contains a pool of PS

biosynthesis loci. As a result, *B. fragilis* exhibits an astonishing diversity of surface polysaccharides (PS); the much-studied PSA of *B. fragilis* strain NCTC9343 is only one of 28 or more different *B. fragilis* polysaccharides [47]. Up to 11 different PS can be variably expressed within an individual strain, and different strains can have a different 'set' of up to 11 PS biosynthesis operons. How many and which of the divergent PS from the *B. fragilis* pan-genome have similar properties to the PSA of NCTC9343 remain to be determined. The diversity of PS produced by different strains of *B. fragilis*, coupled with within strain ON-OFF-ON switching [8], suggests that there is a potentially highly complex dynamic to the interaction of these PS with the immune system. As will be discussed later, PSA was originally shown to rescue T cell deficiency and phenotype skewing in germ-free mice, a discovery that placed the spotlight on microbe-mediated immune development and function.

Microbial Regulation of Immunity in the Gastrointestinal Tract

The microbial system of the GI tract is inextricably linked to the host immune system, with bidirectional communication and influences shaping the repertoire and profiles of both systems. Beyond gastrointestinal homeostasis, it is now well established that gut microbiota influence both innate and adaptive immune development and responses and that this influence extends far beyond immunity in the gut itself [40]. Mechanisms via which microbes skew immune responses are an area of active research currently, with key roles for microbial diversity, modulation of barrier function and microbial metabolites. Consequentially, use of antibiotics which will be discussed in detail later, not only serves to increase the risk of emergence of multi-drug-resistant bacterial strains but can also modulate host immunity via the aforementioned microbial mechanisms.

The gut-associated lymphoid tissue (GALT), similar to other lymphoid tissues, provides hotspots for immune priming, education, activation, modulation and resolution. Surprisingly, the GALT comprises >70% of the total immune system emphasising the importance of this tissue in immune homeostasis and responses [59]. Germ-free mice, limitations notwithstanding, have provided valuable insights to the importance of gut microbiota in immune development, and particularly in relation to GALT. The GALT comprises a range of structures including Peyer's patches, mesenteric lymph nodes and isolated lymphoid follicles. Peyer's patches are specialised raised structures within the gut mucosa primarily comprising epithelial, lymphoid and myeloid cells. These structures characteristically contain cells of both the innate and adaptive immune systems.

Dendritic cells (DCs) are pivotal innate immune cells that lie in the lamina propria beneath the epithelial layer. DCs project processes between epithelial cells to survey gastrointestinal luminal content and take up antigen. DCs also sense microbial components through innate pattern recognition receptors (PRRs) such as Toll-like receptors (TLR), NOD-like receptors (NLR) and RIG-I-like receptors (RLRs) [55, 57]. DCs interact with lymphocytes in Peyer's patches and mesenteric

lymph nodes. In Peyer's patches, DCs that have been exposed to luminal commensal bacteria promote IgA production by B cells which is transported to the lumen to coat the mucosal surface [35]. Within Peyer's patches, DC can present antigen to T cells although DCs also traffic to mesenteric lymph nodes where antigen presentation and activation of T cells are even more prominent and efficient. This serves as a key bridge between innate and adaptive immunity both within the GALT and in the context of overall host immunity.

Epithelial cells of the gut also express PRR which confer the capacity of epithelia to sense and respond to the microbiota. These epithelial cells produce antimicrobial peptides and mucous which promotes barrier function to limit access of pathogens to the submucosa, key host defence mechanisms within the GI tract. Mucous production is diminished in germ-free mice and can be restored with microbial products such as peptidoglycan (PGN) or lipopolysaccharide (LPS) [48], demonstrating the importance of the gut microbiota to barrier function and host defence.

In recent years, the range of adaptive $CD4^+$ T cell subsets has greatly expanded to include T helper (Th)1, Th2, Th9, Th17, Th22, Treg, Tr1 and T follicular helper (Tfh) and continues to expand. Differentiation of these subsets is driven by the cytokine environment which can be directly impacted by the gut microbiota. Indeed, specific types of microbes, including commensal bacteria, are implicated in $CD4^+$ T cell differentiation. Germ-free mice demonstrate Th2 skewing and overall impaired development of lymphoid tissues throughout the body. As discussed earlier, this can be rescued by colonisation with *B. fragilis* or even administration of *B. fragilis* NCTC9343 polysaccharide A (PSA).

Th17 cells, which were first described in 2005 as cellular drivers of autoimmunity [25, 32, 44], are more abundant in the GI tract than elsewhere in the body. Evidence from mice indicates that this is in part due to the presence of segmented filamentous bacteria (SFB) in the terminal ileum which promote Th17 differentiation via IL-6 and IL-23 expression by DCs [19, 27]. SFB are Gram-positive anaerobic bacteria related to the Clostridia. Functionally, Ivanov and colleagues showed that mice lacking SFB and Th17 cells in the gut were more susceptible to pathogenic *Citrobacter rodentium* infection. This study demonstrated a clear promotion of Th17-type adaptive immunity by commensal bacteria in the gut with a consequential benefit to the host of resistance to a pathogenic bacterial threat [27]. These types of studies emphasise the exquisite communication and symbiosis between commensals and immune cells to protect both host and commensal microbes from pathogenic threats.

Regulatory T cells (Treg) are also present in high relatively abundance in the GI tract, particularly in the colonic mucosa. Treg express the transcription factor forkhead box p3 (Foxp3) which is induced by TGF-β signalling. The primary functions of Treg are to induce immune tolerance and resolution of inflammation. Conceptually, it makes sense that such potent modulators of inflammatory responses would be present at the site of constant antigenic challenge; however, the microbial influence in the development and function of Treg in the gut has only recently begun to emerge. As with all T cells, Treg are reduced in the GI tract of germ-free mice. Commensal bacteria such as *Clostridia* spp. have been shown to be crucial to the

development of Treg in colonic mucosa by inducing TGF-β expression to drive Treg differentiation. The role of the microbiota in bridging innate and adaptive immunity can be further exemplified by the influence of *B. fragilis* in Treg development. As described earlier, PSA from *B. fragilis* can rescue T cell deficiency observed in germ-free mice. Colonisation with *B. fragilis* results in uptake of PSA by dendritic cells which are capable of presenting this polysaccharide via MHC Class II to $CD4^+$ T cells [10]. DCs exposed to PSA also produce pro-inflammatory cytokines such as IL-12 and TNF-α via TLR2 signalling. Interestingly, however, *B. fragilis* has also been shown to induce IL-10-producing Treg, particularly in models of overt inflammation (e.g. TNBS-induced colitis, adoptively transferred T cell-induced colitis) [36, 52]. In this setting, TLR2 signalling in T cells was required for anti-inflammatory IL-10 expression but TLR2 expression in DCs was dispensable. Most likely, the development of immunosuppressive Treg facilitates the colonisation by *B. fragilis* whilst also inhibiting the development of autoimmune colitis, another example of symbiotic microbial-mediated regulation of immunity.

The microbiota also produce metabolites that influence host immune function. In particular, short-chain fatty acids (SCFA) such as butyrate, acetate and propionate which bacteria generate from dietary fibre influence barrier function and immune responses [58]. In the context of Treg development, microbial metabolites have also been implicated as mechanisms of commensal-induced Treg differentiation. Arpaia and colleagues demonstrated that butyrate and propionate induced the differentiation of peripheral Treg in mice which may be due to histone deacetylase inhibition [2]. At the same time, Furusawa and colleagues also observed Treg expansion in response to butyrate via enhanced histone H3 acetylation of the Foxp3 locus and demonstrated that these cells could ameliorate experimental colitis [18].

Microbiota in the gastrointestinal tract have also been shown to influence immune function at distant anatomical sites, even as distant as the brain. Microglia are macrophage-like cells resident in the central nervous system (CNS) that are derived from the yolk sac and are self-renewing [23]. Microglia express a range of PRR and can respond to danger signals in the CNS [33] though the relative contribution of microglia versus recruited monocytes/macrophages is not clear in most settings. Under 'resting' conditions, microglia display a ramified morphology and constantly sense their immediate environment. Erny and colleagues recently demonstrated that the maturation and function of microglia are intrinsically linked to the host microbiota as germ-free mice or adult mice depleted of bacteria (prolonged broad-spectrum high-dose antibiotic administration) demonstrated diminished microglial numbers and maturation phenotype with altered cell morphology. This immature phenotype could be rescued either by recolonisation or by administration of short-chain fatty acids, suggesting that bacterial metabolism in the gut can modulate microglial function in the brain [15]. Elucidating such gut-immune-brain communication may help to unravel mechanisms underlying the range of behavioural and neurological functions that have been shown to be influenced by gut microbiota [1, 5, 49, 53].

Although the majority of research on gut microbiota-mediated immune modulation has centred on bacteria, Kernbauer and colleagues recently proposed that

murine norovirus (MNV) can confer beneficial functions to the host in the absence of bacteria, using germ-free and antibiotic-depleted mouse models. These benefits were associated with a type I interferon (IFN) host response, exemplifying the functional bidirectional communication between host and microbe [31]. Unravelling the host virome will facilitate the types of studies necessary to understand how different categories of microbes coordinate host immune modulation. Owing to these diverse influences on immune responses, the gut microbiota modulate host defence capacity/efficiency as well as susceptibility to immune-mediated disease such as allergy and autoimmunity. As such, modulation of gut microbes and/or their metabolites may serve as a clinically relevant therapeutic modality to modulate host immune functions in a range of settings. Understanding the roles and mechanisms of microbes and immune populations in such conditions will be vital to therapeutic exploitation.

Antibiotics and the Impact on Host Microbiota

The intimate interaction of the complex community of gut microorganisms is inextricably linked to human health and disease where studies now demonstrate that modulations of this microbial population directly impact on human physiology in disease. This microbial community is key in its protection of the human host from colonisation of enteric pathogens [24], liberating nutrients from food [37] and in signalling immune system regulation [34]. Significant perturbation of the gut microbiome is an inevitable consequence of antibiotic use where the ramifications of antibiotic use extend beyond changes in population structure at both the taxonomic and genomic levels but also extend impact on the functional capacity of the microbiota with rapid persistent effects [22]. Additionally, some studies now suggest that the perturbation of these microbial communities can also affect the metabolism of xenobiotic compounds, e.g. drugs. The changes within the gut microbiota composition can often be asymmetric where bacteria which have higher drug susceptibility are more likely killed. Furthermore, even amongst the survivors, fitness is variable and dependent on whether other species lost to antibiotic treatment are necessary for viability [56, 64].

Regardless of concentration levels, e.g. inhibitory versus subinhibitory, antibiotic exposure can still exert differential effects on the microbiome [16, 22]. At inhibitory antibiotic concentrations, the community structure is disrupted significantly thus promoting the proliferation of pathogenic colonisers or gene transfer of antimicrobial resistance genes [39]. In contrast, at subinhibitory concentrations, antibiotics can function as signalling molecules that may shape and confer stability to the microbial communities [39]. The modulations of host gut microbiota mediated by antibiotic exposure also extend to the type of antibiotic being administered [29, 30]. A study by Jakobsen et al. [28, 30] aimed at elucidating the effects on microbial composition of pharyngeal and faecal taxonomic composition following exposure to clarithromycin, metronidazole and omeprazole demonstrates that broad-spectrum agents have the longest lasting effects on community composition.

Another study shows that the use of ciprofloxacin is generally linked to the depletion of Ruminococcaceae [41]; however, as with other studies, individualistic changes, e.g. timing of drug exposure, age of host, linked to community recovery exist. Accordingly, a study in neonatal mice has linked early life antibiotic exposure in altered composition of intestinal microbiota, thus eliciting effects on relative body mass, absorption of short-chain fatty acids by intestinal microbiota and hepatic fatty acid metabolism [9]. The perturbation of gut microbiota also paves the way for colonisation by pathogenic bacteria as has been shown for *Salmonella Typhimurium* serovar *enterica* and *Clostridium difficile*. In a healthy microbiota, the commensals prevent pathogen proliferation by outcompeting virulent microbes for space and nutrients and by inducing host defences within the colonic epithelium [6, 17]. Antibiotic-related disruption of the relative composition of the commensal community can reduce the host defences [43] or increase nutrient availability in the absence of certain scavenging commensals, thus promoting pathogen proliferation.

There is a clear correlation between antibiotic use and the selection of resistance genes which, when present, confer a survival advantage that serves to mitigate the fitness costs associated with antibiotic resistance. Hence, it is evident that the gut microbiome can serve as an excellent reservoir for resistance genes. Rapid genetic exchange is facilitated by the array of horizontal gene transfer techniques, which include conjugation, phage transduction and natural transformation. A study by Jakobsson et al. [28, 30] demonstrated that clarithromycin regimen for the treatment of *H. pylori*-related peptic ulcers resulted in a 1000-fold increase of *ermB*-encoding resistance genes after treatment. Antibiotic treatment provides the basis for perturbation of the microbial population, but antibiotic-induced horizontal gene transfer can also fortify the genomic repertoire of the microbiota to endure stress and maintain its functional contributions towards host health. With the increasing emergence of antibiotic resistance, it will be important to maintain surveillance of, and research into the, direct and indirect impact of antibiotic use on immunity and human health.

Closing Remarks

Despite extensive knowledge of both immunological and microbiological aspects of gastrointestinal physiology, many fundamental questions require further research. For example, what differentiates whether a microbe becomes a commensal versus a pathogen? It is simply a matter of location, location, location? Are there microbial triggers that differentiate between friend and foe, such as secretion systems? Can skewing of immune responses by extrinsic factors such as diet, obesity or medications skew gut immunity to enable an altered environment in which new microbes thrive? The converse is also important to ask – how do alterations in gut microbiota influence systemic immune responses and even those at sites as distal as the brain? Addressing these questions will require interdisciplinary teams and creative approaches that cross traditional boundaries but holds potential to advance our knowledge immeasurably and improve human health.

References

1. Arebi N, Gurmany S, et al. Review article: the psychoneuroimmunology of irritable bowel syndrome – an exploration of interactions between psychological, neurological and immunological observations. Aliment Pharmacol Ther. 2008;28(7):830–40.
2. Arpaia N, Campbell C, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013;504(7480):451–5.
3. Arumugam M, Raes J, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473(7346):174–80.
4. Baughn AD, Malamy MH. The strict anaerobe *Bacteroides fragilis* grows in and benefits from nanomolar concentrations of oxygen. Nature. 2004;427(6973):441–4.
5. Bercik P, Collins SM. The effects of inflammation, infection and antibiotics on the microbiota-gut-brain axis. Adv Exp Med Biol. 2014;817:279–89.
6. Brandl K, Plitas G, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008;455(7214):804–7.
7. Bry L, Falk PG, et al. A model of host-microbial interactions in an open mammalian ecosystem. Science. 1996;273(5280):1380–3.
8. Cerdeno-Tarraga AM, Patrick S, et al. Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. Science. 2005;307(5714):1463–5.
9. Cho I, Yamanishi S, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature. 2012;488(7413):621–6.
10. Cobb BA, Wang Q, et al. Polysaccharide processing and presentation by the MHCII pathway. Cell. 2004;117(5):677–87.
11. Drasar BS, Duerden BI. Anaerobes in the normal flora of man. In: Duerden BI, Drasar BS, editors. Anaerobes in human disease. London: Edward Arnold; 1991. p. 162–79.
12. Duncan SH, Hold GL, et al. Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. nov. Int J Syst Evol Microbiol. 2002;52(Pt 6):2141–6.
13. Duncan SH, Louis P, et al. Cultivable bacterial diversity from the human colon. Lett Appl Microbiol. 2007;44(4):343–50.
14. El Aidy S, van den Bogert B, et al. The small intestine microbiota, nutritional modulation and relevance for health. Curr Opin Biotechnol. 2015;32:14–20.
15. Erny D, Hrabe de Angelis AL, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965–77.
16. Finley RL, Collignon P, et al. The scourge of antibiotic resistance: the important role of the environment. Clin Infect Dis. 2013;57(5):704–10.
17. Fukuda S, Toh H, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011;469(7331):543–7.
18. Furusawa Y, Obata Y, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504(7480):446–50.
19. Gaboriau-Routhiau V, Rakotobe S, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity. 2009;31(4):677–89.
20. Gaskins HR. Immunological aspects of host/microbiota interactions at the intestinal epithelium. In: White BA, Isaacson RE, Mackie RI, editors. Gastrointestinal microbiology, vol. 2. New York: Chapman and Hall; 1997. p. 537–87.
21. Gibson GR, MacFarlane GT. Human health: the contribution of microorganisms. In: Gibson SAW, editor. Intestinal bacteria and disease. London: Springer; 1994. p. 53–62.
22. Gibson MK, Pesesky MW, et al. The yin and yang of bacterial resilience in the human gut microbiota. J Mol Biol. 2014;426(23):3866–76.
23. Ginhoux F, Greter M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010;330(6005):841–5.
24. Haag LM, Fischer A, et al. Intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogate colonization resistance against *Campylobacter jejuni* in mice. PLoS One. 2012;7(5):e35988.

25. Harrington LE, Hatton RD, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6(11): 1123–32.
26. Henriksson AE, Blomquist L, et al. Small intestinal bacterial overgrowth in patients with rheumatoid arthritis. Ann Rheum Dis. 1993;52(7):503–10.
27. Ivanov II, Atarashi K, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139(3):485–98.
28. Jakobsson HE, Jernberg C, et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One. 2010;5(3):e9836.
29. Jernberg C, Lofmark S, et al. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007;1(1):56–66.
30. Jernberg C, Lofmark S, et al. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology. 2010;156(Pt 11):3216–23.
31. Kernbauer E, Ding Y, et al. An enteric virus can replace the beneficial function of commensal bacteria. Nature. 2014;516(7529):94–8.
32. Langrish CL, Chen Y, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005;201(2):233–40.
33. Lee H, Lee S, et al. Toll-like receptors: sensor molecules for detecting damage to the nervous system. Curr Protein Pept Sci. 2013;14(1):33–42.
34. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011;474(7351):298–306.
35. Massacand JC, Kaiser P, et al. Intestinal bacteria condition dendritic cells to promote IgA production. PLoS One. 2008;3(7):e2588.
36. Mazmanian SK, Round JL, et al. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008;453(7195):620–5.
37. McNeil NI. The contribution of the large intestine to energy supplies in man. Am J Clin Nutr. 1984;39(2):338–42.
38. Miquel S, Martin R, et al. *Faecalibacterium prausnitzii* and human intestinal health. Curr Opin Microbiol. 2013;16(3):255–61.
39. Modi SR, Collins JJ, et al. Antibiotics and the gut microbiota. J Clin Invest. 2014;124(10): 4212–8.
40. Molloy MJ, Bouladoux N, et al. Intestinal microbiota: shaping local and systemic immune responses. Semin Immunol. 2012;24(1):58–66.
41. Naeem S, Li S. Biodiversity enhances ecosystem reliability. Nature. 1997;390:507–9.
42. Namavar F, Theunissen EB, et al. Epidemiology of the *Bacteroides fragilis* group in the colonic flora in 10 patients with colonic cancer. J Med Microbiol. 1989;29(3):171–6.
43. Ng KM, Ferreyra JA, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature. 2013;502(7469):96–9.
44. Park H, Li Z, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6(11):1133–41.
45. Patrick S. *Bacteroides*. In: Boulnois B, Griffin G, Hormaeche C, Keusch G, Levine M, Smith H, Williams P, Sussman M, editors. Molecular Medical Microbiology, vol. 3. London: Academic Press; 2002. p 1921–48.
46. Patrick S. *Bacteroides*. In: Tang Y-W, Sussman M, Poxton I, Liu D, Schwartzman J, editors. Molecular Medical Microbiology, vol. 2, Second Edition. London: Academic Press; 2015. p 917–44.
47. Patrick S, Blakely GW, et al. Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of *Bacteroides fragilis*. Microbiology. 2010;156(Pt 11):3255–69.
48. Petersson J, Schreiber O, et al. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. Am J Physiol Gastrointest Liver Physiol. 2011;300(2):G327–33.
49. Petra AI, Panagiotidou S, et al. Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. Clin Ther. 2015;37(5):984–95.
50. Poxton IR, Brown R, et al. Mucosa-associated bacterial flora of the human colon. J Med Microbiol. 1997;46(1):85–91.

51. Qin J, Li R, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65.
52. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107(27):12204–9.
53. Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe. 2015;17(5):565–76.
54. Schenk M, Bouchon A, et al. Macrophages expressing triggering receptor expressed on myeloid cells-1 are underrepresented in the human intestine. J Immunol. 2005;174(1): 517–24.
55. Schmolke M, Patel JR, et al. RIG-I detects mRNA of intracellular *Salmonella enterica* serovar Typhimurium during bacterial infection. MBio. 2014;5(2):e01006–14.
56. Sommer MO, Dantas G. Antibiotics and the resistant microbiome. Curr Opin Microbiol. 2011;14(5):556–63.
57. Strober W. The multifaceted influence of the mucosal microflora on mucosal dendritic cell responses. Immunity. 2009;31(3):377–88.
58. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev. 2001;81(3):1031–64.
59. Vighi G, Marcucci F, et al. Allergy and the gastrointestinal system. Clin Exp Immunol. 2008;153 Suppl 1:3–6.
60. Walker AW. The human microbiota and pathogen interactions. In: Debanne SM, Tang Y-W, Liu D, Poxton IR, Schwartzman JD, editors. Molecular medical microbiology, vol. 2. London: Academic; 2015. p. 347–56.
61. Walker AW, Duncan SH, et al. Phylogeny, culturing, and metagenomics of the human gut microbiota. Trends Microbiol. 2014;22(5):267–74.
62. Wesolowska-Andersen A, Bahl MI, et al. Choice of bacterial DNA extraction method from fecal material influences community structure as evaluated by metagenomic analysis. Microbiome. 2014;2:19.
63. Wexler HM. *Bacteroides*: the good, the bad, and the nitty-gritty. Clin Microbiol Rev. 2007; 20(4):593–621.
64. Willing BP, Russell SL, et al. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol. 2011;9(4):233–43.
65. Willis AT. Abdominal sepsis. In: Duerden BI, Drasar BS, editors. Anaerobes in human disease. London: Edward Arnold; 1991. p. 197–223.

Chapter 4
Roles of Substance P in Gastrointestinal Functions and Neuroimmune Interactions

Janek Vilisaar and Razvan I. Arsenescu

Abstract Substance P (SP), a member of the tachykinin (TK) peptide family, is ubiquitously expressed from invertebrates to mammals with a role in different organ systems and a number of functions also in the gastrointestinal (GI) tract. In the current chapter, the roles of SP and other TKs in the GI tract with wider implications and its role in intersystem communication are emphasized. Roles for SP and different neurokinin receptors in the enteric nervous system and neuroimmune modulation are covered. In disease, SP roles in autoimmune and other inflammatory and infectious conditions are summarized.

Keywords Substance P • Tachykinins • Neurokinin receptors • NK1 receptor • Truncated NK1 receptor • Inflammation • Neuropeptides

Abbreviations

ACh	Acetylcholine
CD	Crohn's disease
CGRP	Calcitonin-gene-related peptide
ChAT	Choline acetyltransferase
CNS	Central nervous system
EKA	Endokinin A
EKB	Endokinin B
EKC	Endokinin C
ENS	Enteric nervous system
ERK1/2	Extracellular signal-regulated kinases 1 and 2
GI tract	Gastrointestinal tract

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HK-1	Hemokinin-1
IBD	Inflammatory bowel disease
ICC	Interstitial cell of Cajal
IPAN	Intrinsic primary afferent neuron
mRNA	Messenger ribonucleic acid
NFκB	Nuclear factor-κB
NK1R	Neurokinin-1 receptor
NK1R-T	Truncated neurokinin-1 receptor
NKA	Neurokinin A
NKB	Neurokinin B
NPK	Neuropeptide K
NPγ	Neuropeptide-γ
SP	Substance P
TKs	Tachykinins
UC	Ulcerative colitis
VIP	Vasoactive intestinal peptide

Introduction

Substance P (SP) is conventionally regarded as a neurotransmitter in pain pathways and has a role in neurogenic inflammation (with effects of vasodilatation and plasma extravasation), in motility of different organ tracts, secretory function, and as a mediator of autonomic reflexes, such as vomiting. SP is a transmitter and modulator in the enteric nervous system (ENS) and sensory nerve fibers that innervate other organs, including spleen, thymus, and lymph nodes. Neuroimmune communication is one of the more recently recognized facets of SP. It mediates immune functions via different mechanisms, including inflammatory cytokine induction, T- and B-cell proliferation and differentiation, T-helper phenotype commitment, chemotaxis, and adhesion molecule expression. The role of SP has been shown in different autoimmune conditions, such as inflammatory bowel disease as well as hepatitis, pancreatitis, and GI infections with corresponding differential effects. SP and its NK1-receptor (NK1R) isoforms are widely expressed in different cell types, including enteric neurons, glia, various immune cells, mast cells, endothelial cells, and different intestinal cells, such as epithelial, enteroendocrine, and smooth muscle cells [1]. The role for SP has been additionally attributed in tumor cell proliferation, angiogenesis, migration, and infiltration with the involvement of NK1R-truncated isoform in chronic inflammation and transition to malignancy [2]. Roles mediating hepatotoxicity and cell survival of SP have been recognized, and more recently, its role in ENS plasticity has been suggested [3, 4].

Importantly, SP, as part of the tachykinin (TK) peptide family, represents merely one of the TK peptides in the GI tract. Functions of TKs are closely interlinked with

numerous other mediators in the gut. This chapter focuses mainly on SP and its interactions predominantly in the human and mouse systems.

Substance P in the Family of Tachykinins

TKs are a family of peptides, sharing the common C-terminal amidated amino acid region, -Phe-X-Gly-Leu-Met-NH_2, where X is either an aromatic or a β-branched aliphatic amino acid. This is the minimal sequence for exerting biological activity at the TK receptors [1]. Aromatic phenylalanine or tyrosine in this location is considered indicative of NK1R binding affinity [5]. The key member of the TK family, SP, is an 11-amino acid peptide with the sequence Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2.

The other members, neurokinin A (NKA) neurokinin and B (NKB), together with SP, were first found to be expressed in sensory neurons (both central and enteric); hence they have been conventionally regarded as neuropeptides, and other members of the family exist, such as the elongated forms of NKA, i.e., neuropeptide K (NPK) and neuropeptide-γ (NPγ), as well as a more recently discovered hemokinin-1 (HK-1), with its two known elongated forms, endokinin A (EKA) endokinin and B (EKB).

Three genes encoding TKs have been identified in humans: TAC1 (or PPT-I or PPT-A), TAC3 (or PPT-II or PPT-B), and TAC4 (or PPT-C). TAC1 consists of seven exons; the sequence that encodes SP is contained within exon 3. Alternative splicing of TAC1 transcript gives four distinct, α, β, γ, and δ, forms of messenger ribonucleic acid (mRNA). α-TAC1 and δ-TAC1 encode SP; β-TAC1 encodes SP, NKA, and NPK; and γ-TAC1 encodes SP, NKA, and NPγ [6]. The TAC3 gene transcribes three forms of mRNA (α, β, γ) of which only the α- and β-TAC3 are translated into NKB [7]. The TAC4 gene can be transcribed into α, β, γ, and δ mRNA isoforms of which the α isoform has two variants: α-TAC4v1, encoding EKA and EKC, and α-TAC4v2, encoding EKB and EKC. The EKC has a C-terminal Leu-NH2 and cannot be properly considered a TK; it also lacks significant activity at the TK receptors.

SP and other TKs are expressed in different cell types: different types of neurons, in both myenteric and submucosal ganglia, as well as dorsal root and vagal ganglia, glial cells, peripheral immune cells, endothelial cells, and intestinal cells, such as epithelial cells and enterochromaffin cells [1, 8]. TK containing nerve fibers surround both myenteric and submucosal ganglia and blood vessels, ramify through muscle, and supply the mucosa, forming networks beneath the epithelium [8, 9].

Regarding neuronal expression of TKs, SP and NKA have been shown in intrinsic primary afferent neurons (IPANs) of the intestine and their excitatory synapses with other neurons, in ascending myenteric interneurons as well as in myenteric excitatory motor neurons, regulating both longitudinal and circular muscle activities [1, 10–12]. However, in the efferent-motor response, nitric oxide (NO) and vasoactive intestinal peptide (VIP), rather than TKs and/or CGRP, are important

[13, 14]. Works detailing different localizations of TK-like immunoreactivity in the GI tract are well summarized in the review by Lecci [1].

Substance P Receptors

TKs exert their effects on target cells through the TK receptor family. These receptors consist of seven hydrophobic transmembrane domains, connected by extra- and intracellular loops and are coupled to G-proteins [15–17]. The three main receptors, neurokinin-1 (NK1), NK2, NK3 receptors (also NK1R, NK2R, NK3R), are widely expressed in the GI tract. All TKs show some degree of cross-reactivity among these receptors as their affinity is dictated by the common C-terminal amino acid sequence. NK1, NK2, and NK3 receptors are exhibiting preferences for substance P, neurokinin A, and neurokinin B, respectively [18]; more specifically, these affinities can be expressed as follows: SP = hHK-1 > NKA > NKB for NK1R, NKA > NKB > SP > hHK-1 for NK2R and NKB > NKA > hHK-1 > SP for NK3R [19].

NK1 receptors are expressed in enteric neurons, glia, and different effector cells, such as smooth muscle cells of both longitudinal and circular layers, muscularis mucosa and interstitial cells of Cajal (ICC) [1, 20–24], epithelial cells, and endothelial and peripheral immune cells, including mucosal mononuclear cells, eosinophils, and mast cells [1]. Neuronal localization of NK1 receptors has been detected in the submucosal plexus (cell bodies of secretomotor neurons and IPANs) and myenteric plexus (cell bodies of IPANs, ascending interneurons, inhibitory and excitatory motor neurons) [1, 10].

NK2 receptors are mostly expressed in circular and longitudinal smooth muscle cells, muscularis mucosa, epithelial cells, and possibly enterochromaffin cells [1, 10, 23, 25]. NK2 receptors can be also expressed in nerve terminals of cholinergic (and TK expressing) excitatory motor neurons in humans [1]. They mediate excitatory neuroeffector function regarding motility and secretory activity [1]. *In vitro* experiments have shown that NK2 receptors play a more prominent neuroeffector role in non-adrenergic, non-cholinergic excitatory transmission with effects on human intestinal smooth muscle cells [1]. Although some expression of NK3 receptors in ICC and smooth muscle of the human esophagus has been shown, the expression of NK3 receptors in the GI tract is mostly neuronal (both myenteric and submucosal plexus) [1].

Two isoforms of NK1R exist in humans with a different length of the C-terminal end: a full-length NK1R (also referred to as NK1R-F), consisting of 407 amino acids, and a C-terminally truncated isoform (NK1R-T), consisting of 311 amino acids [26–29]. Limited evidence exists on the distribution of these isoforms in different tissues. NK1R-T has been found in peripheral tissues, including cells of monocyte lineage [28, 29] and some discrete brain regions (cortex, cerebellum), whereas NK1R is predominant in the rest of the CNS [30, 31] and ubiquitously elsewhere. NK1R-T has been also reported in colonic epithelial cells of patients with colitis-associated cancer [2, 8].

Full-length NK1R has been found to stimulate multiple second messenger systems and couple to several members of the G-protein family (Gq/11, Gαs, Gα0) [32, 33]. G-proteins are coupled to the third cytoplasmic loop of the NK1R [34]. As receptor agonists can stabilize distinct receptor conformations, individual TKs may signal differently via the same NK receptors with diverse outcomes [8]. Upon receptor-ligand interaction, conformational changes within the receptor cause exchange of guanosine diphosphate (GDP) with guanosine triphosphate (GTP) that activates the attached G-protein. The α-subunit of the G-protein dissociates from the β- and γ-subunits and activates the intracellular effectors [35], such as the members of the mitogen-activated protein kinase (MAPK) cascade, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38MAPK [36, 37]. ERK1/2 translocate into the nucleus and mediate phosphorylation of transcription factors, such as NFκB, widely involved in inflammatory functions [38, 39]. p38MAPK subfamily comprises kinases functioning as a signal transduction pathway, independent of NFκB. This pathway also has been shown to mediate SP-induced inflammatory cytokine expression [37]. Another effector of G-protein activation is phospholipase Cβ (PLCβ) that hydrolyses phosphatidylinositol biphosphate (PIP2) into two second messenger molecules, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 acts on specific receptors to release intracellular stores of calcium. DAG acts via protein kinase C (PKC) to open calcium channels leading to relevant tissue responses.

TK receptor activation can be coupled via G-proteins also to adenylyl cyclase activation [35] that converts ATP into cAMP. The second messenger cAMP acts via different effectors involving crosstalk between different signaling pathways [40].

Little is known to date about NK1R-T signaling. NK1R-T has not been shown to induce calcium mobilization independently, but as a result of crosstalk with coreceptor-mediated responses, such as chemokine receptor CCR5 [28]. NK1R-T is not phosphorylated and does not interact with β-arrestins whereby it is defective in desensitization and endocytosis [8, 41, 42], which are important processes in NK1R signaling. ERK1/2 pathway has been suggested in NK1R-T downstream signaling [28].

Substance P in Gastrointestinal Physiology

TKs in the ENS play a role primarily in excitatory neurons, alongside with acetylcholine (ACh), serotonin, and many other transmitters. Mainly NK1 and NK3 receptors mediate tachykininergic neuro-neuronal transmission. Similar levels of SP and NKA are contained in mucosal and muscle layers of the human intestine [1, 43], and they can be co-released by excitatory motor neurons upon adequate stimuli [1, 44]. NK1 and NK2 receptors are expressed in both layers of the intestinal smooth muscle [1], and both SP and NKA have been reported regulating motility, secretion, and vascular and immune functions [7].

This section focuses on the role of SP and other TKs in motility, secretory activity, and autonomic reflexes. SP role in peripheral immunoregulation will be covered in a separate section on immunomodulatory effects.

Motility

TKs are important peptide regulators of intestinal motility at various levels; they are expressed in cholinergic excitatory motor neurons, projecting to both circular and longitudinal muscles, interneurons, and intramural and extramural sensory neurons [1, 34]. TK effects on motility depend greatly on the GI tract segment and effects of other mediators in the interplay with the autonomic system [45]. A number of different agents co-mediate TK signals. SP has been shown to contract all parts of the GI tract in mammals [46, 47]; the effects are predominantly mediated via NK2R, inducing excitation and contraction [47], although NK1R also play a role [47–49].

Chemical or mechanical stimulation of the mucosa or distention of the muscle excites primary sensory neurons within the myenteric plexus, which release TKs (SP, NKA) and activate ascending excitatory and descending inhibitory motor pathways [7]. TKs acting via NK3R contribute to transmission from ascending interneurons to excitatory motor neurons, whereas transmission to inhibitory motor neurons involves NK1R [7]. In many instances, particularly in the ascending excitation of the circular muscle, TKs synergize with ACh [7].

SP together with NKA facilitates motor activity via NK1R on ICC and via NK2R on smooth muscle [7, 12]. In ICC, SP activates a nonselective cation channel that controls pacemaker functions [50] and Na^+-leak channels that mediate depolarization [51]. In the human colon, SP, NKA, and NKB all can stimulate contraction of circular muscle by activating mainly NK2R on colonic myocytes [8, 52].

Thus, TKs (SP and NKA) cannot only stimulate but have inhibitory effects on motility, the net response depending on the type and site of activated NK receptors [7]. In the human GI motility, predominantly NK2 receptors mediate excitatory effects, although NK1 receptors on smooth muscle play a role, and their contribution to tachykininergic co-transmission may be additive to varying degrees, depending on different stimuli and their duration [1]. NK1 receptors may have a role in relaxation of the contracted muscle [1, 53]. SP and NKA can depress motor activity also through release of inhibitory transmitters, such as nitric oxide (an effect exerted in particular via NK1R and less by NK3R on inhibitory motor pathways) [7].

Secretory Activity

TKs are also involved in the control of secretory activity in the GI tract, although they represent merely one of many active agents [45]. SP and NKA, acting via NK1 and NK3 receptors on enteric neurons, participate in the transmission to secretomotor

neurons, which cause ion secretion through the release of ACh and/or VIP [7]. Additionally, TKs can be released from axon collaterals of intrinsic sensory neurons, close to the epithelial effector cells, and elicit chloride secretion via an axon reflex-type mechanism [7]. TKs act directly on NK1 or NK2 receptors on enterocytes to stimulate chloride and bicarbonate secretion [7].

The knowledge of effects on salivary activity by SP in humans and mice is modest [45]. The principal effect of SP and NKA is to enhance the flow of salivary fluid, which is poor in protein, via a predominant action on the secretory structures of the acini [45]. SP affects the output of salivary components, secretion of K^+ and Cl^- ions, and discharge of proteins, glycoproteins, proteolytic enzymes, amylase, kallikrein, and mucus [10, 45]. This largely varies between different species and different glands (sublingual, parotid, etc.).

In gastric acid and biliary secretion, effects of cholecystokinin, VIP, and other stimuli are more important [45]. In intestinal secretion, predominantly NK1 and NK2 receptors are participating [45]. SP is involved in secretion of electrolytes and water into the lumen of the small intestine [47] and colon [54]. In pancreatic secretion, the effects of TKs are negligible in comparison with that of cholecystokinin [45].

With the increase in the capillary permeability and other inflammatory effects in pathological states, TKs may more importantly contribute to hypersecretory, vascular, and immunological disturbances in these circumstances [45].

Autonomic Reflexes

A number of autonomic reflexes, including vomiting, swallowing, peristaltic reflexes, visceromotor reflex, e.g., to colorectal distension, and cough, and cardiovascular and other reflexes are suggested to be mediated by SP; however, the mechanisms of these reflexes remain incompletely understood. One of the best studied is a vomiting reflex, a somatoautonomic reflex where SP, alongside with serotonin, glutamate, and other mediators, has been attributed a role [55]. The circuitry of the vomiting reflex is complex. The individual sensory pathways (e.g., vagal, vestibular, etc.) are well understood. The central role is attributed to the nucleus of the solitary tract of the vagus nerve and nearby nuclei in the medulla, including area postrema, where SP has a role, and a number of afferent projections (cerebral, vestibular, gut afferent inputs) [56]. However, their integration, the central pattern generator, and the final common (efferent) pathway are poorly delineated [56, 57].

Many factors, peripheral, central, and systemic, are known to cause vomiting. The roles for SP and NK1R in the vomiting reflex are thought to involve the afferent pathway [55], as well as centrally the induction of emesis and effects on somatomotor responses [58–61]. Less is known on SP role in simultaneously occurring autonomic responses, such as salivary excretion, associated with emesis [61]. Very scanty evidence exists on other NK receptor subtypes, other than NK1R, mediating emesis.

Some information on the role of SP in emesis comes from preclinical trials using selective NK1R antagonist, aprepitant (MK-869), which centrally inhibits emesis, induced by cytotoxic chemotherapeutic agents [62, 63]. Clinical evidence has accumulated since it was licensed in 2003 with an indication for chemotherapy-related and postoperative nausea and vomiting. Aprepitant and its intravenously used prodrug, fosaprepitant, have been shown to have a broader spectrum of antiemetic effects (both acute and delayed emeses), compared to 5-HT_3 receptor antagonists [64]. The NK1R antagonist effects will be further discussed in the section on therapy.

Inflammatory and Immunomodulatory Effects of Substance P

The function of SP and other neuropeptides in sensory nerves is not confined to mediating sensation. TK-containing afferent nerve fibers innervate most organs, including the viscera and primary and secondary lymphoid organs [45, 65, 66]. Release of TKs from the nerve endings is exerting various effects on these tissues; in addition, different immune cells (lymphocytes, cells of monocyte lineage, dendritic cells, eosinophils, etc.) express TKs and their receptors (Fig. 4.1). This provides means via which SP-mediated neural control over immune responses as well as reciprocal communication is implemented. Numerous mediators, such as cytokines, and their crosstalk with neuropeptides are important in creating a particularly inclined neuroimmune milieu.

The GI system, also considered the largest lymphoid organ of the body, is rich in peptidergic innervation and SP content [68]. Mucosal neuroimmune interactions are important in modulating gut homeostasis and pathophysiology [68, 69]. Regarding NK1 receptors, most of the knowledge has been obtained on full-length NK1R; however, recently, the role of NK1R-T has been shown with some more evidence to date. Both full and truncated NK1R isoform expressions can be seen in human mucosal mononuclear cells, suggesting a role for SP in mucosal immunomodulation [34, 68].

Immunoregulatory effects of SP are exerted to a great extent via its modulation of cytokine production by different types of cells. SP has been shown to stimulate IL-1, IL-6, IL-8, IL-10, IL-12, and TNF-α production from monocytes and macrophages [70–73] and IL-2, IFN-γ, IL-4, and IL-10 from T cells [74, 75]. In glial cells, SP can stimulate IL-6 and IL-1 production [76–79]. IL-12 and IL-23 induction by SP in peripheral blood mononuclear cells has also been shown [67].

In infectious models of the GI tract, different immune regulatory circuits have been described. IFN-γ production in T cells has been shown to be mediated via NK1R, suggesting a role for SP in the granulomatous response, such as shown in the *Schistosoma mansoni* model [75]. SP enhances IFN-γ production in different inflammatory settings [75, 80] and as part of the IL-12 immune regulatory circuit may be important in promoting Th1 responses [81, 82]. Additionally, other TKs, such as hemokinin-1 (HK-1), have been suggested in different GI conditions [82].

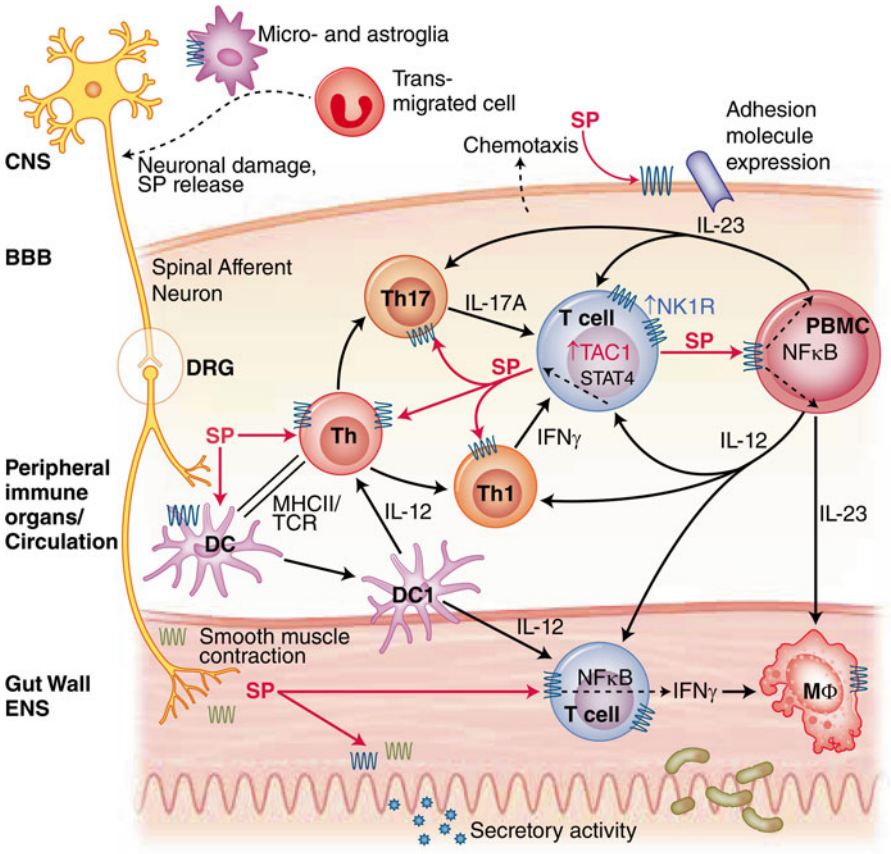

Fig. 4.1 Schematic diagram illustrating neuroimmune interactions of substance P (SP) and associated cytokines in primarily cellular immune responses, suggested in inflammatory pathways in the gastrointestinal tract and central nervous system (*CNS*). For clarity, only the main pro-inflammatory effects are depicted, referring to the human system. *Blue lines* indicate NK1R (neurokinin-1 receptor) and *green lines* NK2R (neurokinin-2 receptor). SP effects are shown in *red*. Secretory activity in GI mucosa is mediated via NK1R and NK2R and contraction of smooth muscle predominantly via NK2R. *BBB* blood-brain barrier, *DC* dendritic cell, *DRG* dorsal root ganglion, *ENS* enteric nervous system, *MΦ* macrophage, *PBMC* peripheral blood mononuclear cell, *MHCII* major histocompatibility complex II, *TCR* T-cell receptor, *Th* T helper cell (Adapted from Ref. [67])

Other effects attributed to SP include stimulating proliferation of T and B cells [83–85]. SP is known to act as a B-cell differentiation cofactor and has effects on immunoglobulin production [85, 86]. SP enhances cell-mediated cytotoxicity of T-cytotoxic and natural killer cells [87] as well as phagocytosis in macrophages [88] with inducing oxidative burst and release of oxygen radicals and arachidonic acid derivatives mediating tissue injury and indirectly stimulating recruitment of immune cells [83].

The mechanisms of chemotaxis by SP are not clear. Evidence exists that the chemotactic activity may reside in its C-terminal amino acid sequence [34].

Chemoattractant effect of SP on neutrophils has been described [89] and loss of it in NK1R knock-out mice [90]. SP has also been found to induce macrophage inflammatory protein-1β expression in human T cells [91]. It has been shown that SP promotes CCR5-mediated chemotaxis of human monocytes, which is implemented via crosstalk between CCR5 and NK1R-T on monocytes [28].

SP is involved in leukocyte adhesion to endothelial cells and has been reported to induce the expression of endothelial-leukocyte adhesion molecule-1 on human small vessel endothelium, increase the expression of the leukocyte integrin CD11b on human neutrophils, and enhance the expression of intercellular adhesion molecule-1 and leukocyte function-associated antigen-1 on murine endothelial cells and lymphocytes [92, 93].

Reciprocal interactions exist, i.e., induction of SP and its receptor by various Th17- and Th1-type cytokines. In murine models, IL-12 has been shown to induce SP precursor mRNA in macrophages via STAT4 pathway [81] and NK1R expression by both IL-12 and IL-18 stimulation via NFκB [94]. IL-12 and IL-23 have been found to induce SP synthesis in murine T cells and macrophages which can be regulated by IL-10 and TGF-β, respectively [95]. More recently, IL-23 and less prominent IL-12 effects have been shown on SP and its receptor expression in human T cells [67].

Neurogenic Inflammation

Neurogenic inflammation represents a distinct inflammatory response mediated by the release of SP, as well as CGRP, NKA, and other mediators from afferent nerve endings innervating tissues, including blood vessels. The immediate effects of SP are particularly prominent on the vasculature, where SP induces vasodilatation (together with CGRP) [96]; NK1R stimulation on endothelial cells of postcapillary venules results in plasma extravasation and granulocyte infiltration (almost entirely mediated by TKs) [97] and leukocyte adhesion to endothelial cells of venules [98]. SP stimulates mast cells and basophils to release histamine and other mediators, such as leukotrienes [47, 99], the SP releasing activity of which can further contribute to plasma extravasation and edema [45]. The consequence of neurogenic inflammation is also pain that can positively feedback the above effects [45]. Upon inflammatory/noxious stimuli, SP may be also released centrally from spinal afferent neurons, contributing to the central pain mechanisms including central sensitization [100–104].

Substance P in Gastrointestinal Pathology

The immune regulatory roles of SP and other TKs have been recognized in a number of inflammatory conditions of the GI tract, such as inflammatory bowel disease (IBD) as well as autoimmune hepatitis, pancreatitis, diverticulosis, and different GI infections [1, 105]. Additionally, several functional GI disorders have corresponding

associations. Decreased SP expression in chronic constipation has been noted, whereas in irritable bowel syndrome, evidence for or against a pathogenic role of TKs is balanced [1].

Immunoregulatory effects of SP are exerted to a great extent through its modulation of cytokine production with subsequent effects. As briefly mentioned in the previous section, immune regulatory circuits involving SP play a role in inflammatory conditions. One of the effects of SP and NK1R is to enhance T-cell IFN-γ and IL-17 production, amplifying the proinflammatory response [105]. The effect of IL-12 on IFN-γ production in certain disease models can thus be mediated by induction of SP from T cells as an intermediary step. IL-12 and IL-23 are known to induce production of SP in NFκB-dependent manner from T cells and macrophages, respectively, with inhibition by IL-10 in T cells and TGF-β in macrophages [105]. IL-10 and, to a lesser degree, TGF-β also have inhibitory effects on NK1R expression [105]. At the same time, TGF-β blocks NK1R internalization on receptor activation, allowing enhanced SP effects, promoting T-cell IFN-γ and IL-17 secretion [105, 106]. Several cytokines, such as IL-12, IL-18, and TNF-α, induce NK1R expression on T cells [105].

The role for SP is also demonstrated in reactive neural plasticity of the ENS in various GI conditions with an inflammatory component (primarily inflammatory, infectious, degenerative, or malignant). ENS plasticity, in structural terms, involves local tissue hyperinnervation (neural sprouting, neural and ganglionic hypertrophy) next to hypoinnervated areas, switch in the neurochemical code (neurotransmitter/neuropeptide) toward preferential expression of neuropeptides and activation of peripheral glial cells [4, 107–109]. The neuroimmune interactions involved are also mediated by cytokines and neurotrophic factors, released from a variety of sources, including nerve endings, glia, and different inflammatory cells. This results in organ dysfunction (e.g., impaired motility and secretion), neuropathic pain, and/or hypersensitivity [4]. Widespread upregulation of SP has been shown both in nerve fibers and affected tissues and in noninflamed adjacent tissue [4].

Below are some examples of GI conditions, along with the role of SP and its receptor in their pathogenesis.

Inflammatory Bowel Disease

In both ulcerative colitis (UC) and Crohn's disease (CD), upregulated NK1R mRNA expression has been described in colonic mucosal biopsies and particularly in mucosal CD4+ T cells, compared with noninflamed mucosal expression levels [110, 111]. Pro-inflammatory cytokines induce NK1R expression in colonic epithelial cells, suggesting that colonic inflammation may potentiate further SP-induced inflammatory and proliferative effects [34, 112]. Interestingly, mesenteric adipocytes from IBD patients (CD and UC) also have higher NK1R and NK2R expression relative to healthy controls [113]. SP treatment induces expression of the pro-inflammatory cytokine IL-17 in these adipocytes and thus promotes chronic gut inflammation.

SP upregulation has been shown in the rectum and colon of UC, but not in CD [114–116]. On the other hand, an increased density of SP-immunoreactive fibers has been demonstrated in hypervascular lesions of CD and in CD colon [34, 115, 117].

A role for SP has been shown also in experimental models of IBD. Murine models of IBD suggest that SP (and HK-1) helps to promote intestinal inflammation [105], which is IFN-γ driven. Treating these mice with an NK1R antagonist suppresses intestinal IFN-γ production and inhibits inflammation [118].

Of interest, the truncated NK1R isoform has been shown preferentially expressed in colonic epithelial cells in patients with UC who develop colonic carcinoma [8].

Infections of the GI Tract

Changes in the expression of SP and its receptor have been reported in infections [119–121], where SP effects can be detrimental or protective to the host, depending on the pathogen.

In schistosomiasis, SP via NK1R amplifies the IFN-γ response. IFN-γ production by CD4+ cells is upregulated by SP, the latter being produced locally within granulomas. IFN-γ activates macrophages and influences the function of T and B cells, natural killers with the inflammation becoming more damaging to host tissue when IFN-γ production is abolished [105]. This is supported by experiments in T-cell-selective NK1R knockout mice and using NK1R antagonists [122–124].

Similar effects are seen during salmonella gastroenteritis, in which antagonism of NK1R limits Th1 responses with impaired mucosal IFN-γ response and increased susceptibility to infection [125]. SP effects are protective as SP-mediated IFN-γ production activates macrophages and helps to limit dissemination of bacteria [105]. SP may modulate IFN-γ synthesis in salmonella infection via upregulation of macrophage IL-12 production and downmodulation of TGF-β secretion [105].

Increased viral or bacterial burden and accelerated disease progression with impaired natural killer activity have been described in several studies with impaired SP response [126].

In some other infections, SP effects are damaging to the host, such as in *Clostridium difficile* toxin-A-induced enterocolitis [105]. NK1R in this infection seems to mediate mucosal injury [127]. In experimental models, *C. difficile* toxin A causes extrinsic afferents in the rat and mouse ileum to release SP, which via NK1R excites enteric secretomotor neurons and leads to degranulation of mast cells, macrophage and granulocyte activation, hypersecretion, inflammation, and necrosis [7]. Mice pretreated with NK1R antagonist [128] or NK1R knockout mice are protected from toxin-induced enteritis [105, 127].

SP worsens other infections, such as *Cryptosporidium parvum*, *Taenia crassiceps*, and *Trypanosoma brucei* [105]. Also in polymicrobial sepsis, SP may worsen endotoxin injury; TAC1 knockout mice display reduced mortality in these settings because of dampening of the immune response that induces excessive tissue injury

[105, 129]. In these circumstances, NK1R antagonist would be protective [130]. SP has pro-inflammatory effects also in LPS-induced endotoxemia [131, 132].

SP may not be beneficial in HIV infection, in which SP promotes HIV entry and replication in leukocytes [105, 133, 134]. NK1R mRNA expression is significantly downregulated in patients with HIV infection which may contribute to the mucosal abnormality, altered intestinal motility, and GI symptoms associated with HIV [34, 135].

Therapeutic Perspectives

Currently, three high affinity NK1R antagonists have been cleared by FDA for treating chemotherapy and postoperative nausea and vomiting [136, 137]. The oral formulation, aprepitant, was approved in 2003 and its intravenous form fosaprepitant in 2008. In 2015, two new oral NK1R antagonists were introduced: rolapitant and netupitant. Trials with different other indications have failed so far. Knowledge of SP physiology and mechanisms in pathological states are important in developing a targeted therapeutic approach.

The indications for NK1R antagonist use in clinical practice would need to be specific. As from above examples, NK1R antagonism carries different, even opposing effects in different infections. Additionally, using different receptor antagonists, e.g., combined NK1R and NK2R antagonists, may be beneficial in certain situations. This is a clear indication in certain pathologies, e.g., antagonizing motor effects induced by endogenous release of TKs in the small intestine, whereas the blockade of the NK2R may be sufficient to disrupt physiological motor and possibly secretory activity at the colonic level [1].

Generally, in the diseased states, the contribution of TK neurons seems out of balance and there is a shift away from cholinergic toward tachykininergic regulation [7]. This explains why in animal experiments NK receptor antagonists are not very active in the normal gut but are able to correct disturbed motility, hypersecretion, tissue homeostasis, and pain in certain pathological states [7]. Similarly, minor effects of NK receptor antagonists on GI physiological functions have been also observed in healthy human volunteers [1] as other mechanisms seem to compensate for these effects.

A recent small-phase 1B study in HIV patients [138], using aprepitant at 375 mg daily for 2 weeks, has shown that NK1R blockade has immunoregulatory functions in human subjects. Compared to the placebo-treated group, there were a significant decrease in $CD4^+$ programed death-1 receptor-positive cells (PD-1) and decreased plasma levels of SP and activated macrophages (low-soluble CD163). PD-1 and PD-ligand (PD-L) pathway plays a critical role in immune tolerance and the development of autoimmune conditions [139]. Thus, the negative effect of aprepitant on this pathway may be of concern in patients with IBD. On the other hand, increased NK1R and SP expression in these patients would make aprepitant a good treatment option. Furthermore, patients with CD and UC have increased plasma levels of scavenger receptor CD163, consistent with an activated macrophage phenotype.

Anti-TNF treatment induces remission in IBD patients and is associated with normalization of sCD163. Moreover, nausea, vomiting, and abdominal pain are common symptoms in these patients and often determine quality of life independent of the outcome of mucosal healing. Thus, NK1R blockade holds promise as a novel approach to the treatment of IBD.

Conclusions

Various roles for SP and other TKs in the GI tract have been demonstrated to date. Aspects of SP regulation of GI physiology are relatively well studied, showing SP as a co-neurotransmitter in excitatory neurons and as a mediator in several physiological functions (motility, secretion, etc.). Other important facets of SP are revealed in pathological states. SP has a role in neuroimmune intersystem communication and an increasingly recognized role in mediating inflammatory functions (Fig. 4.1). In autoimmune inflammatory settings, regulation of the inflammatory response by different mechanisms has been shown by SP, including cytokine induction, T-helper phenotype effects, and chemotaxis. In this context, co-effects of SP with other mediators are important in determining the inflammatory milieu and the specific effects. This is also true in infections, where different inflammatory effects of SP have been demonstrated with different pathogens. Pro-inflammatory roles of SP are an appealing therapeutic potential for SP/its receptor antagonism in selected autoimmune and infectious conditions of the GI tract, and this could be used by targeting different receptor subtypes. SP has a key role in neurogenic inflammation and pain mechanisms, highly relevant also in GI conditions. Other less well-known functions of SP need clarification, such as its roles in cell survival.

References

1. Lecci A, Capriati A, Altamura M, Maggi CA, Lecci A, Capriati A, et al. Tachykinins and tachykinin receptors in the gut, with special reference to NK2 receptors in human. Auton Neurosci. 2006;126–127:232–49.
2. Gillespie E, Leeman SE, Watts LA, Coukos JA, O'Brien MJ, Cerda SR, et al. Truncated neurokinin-1 receptor is increased in colonic epithelial cells from patients with colitis-associated cancer. Proc Natl Acad Sci. 2011;108(42):17420–5.
3. Koon H-W, Zhao D, Zhan Y, Moyer MP, Pothoulakis C. Substance P mediates antiapoptotic responses in human colonocytes by Akt activation. Proc Natl Acad Sci. 2007;104(6):2013–8.
4. Demir I, Schäfer K-H, Tieftrunk E, Friess H, Ceyhan GO. Neural plasticity in the gastrointestinal tract: chronic inflammation, neurotrophic signals, and hypersensitivity. Acta Neuropathol. 2013;125(4):491–509.
5. Page NM. Hemokinins and endokinins. Cell Mol Life Sci. 2004;61(13):1652–63.
6. Nawa H, Kotani H, Nakanishi S. Tissue-specific generation of two preprotachykinin mRNAs from one gene by alternative RNA splicing. Nature. 1984;312(5996):729–34.
7. Hokfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. J Intern Med. 2001;249(1):27–40.

8. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: contributions to physiological control and the mechanisms of disease. Physiol Rev. 2014;94(1):265–301.
9. Llewellyn-Smith IJ, Furness JB, Murphy R, O'Brien PE, Costa M. Substance P-containing nerves in the human small intestine. Gastroenterology. 1984;86:421–35.
10. Holzer P, Holzer-Petsche U. Tachykinin receptors in the gut: physiological and pathological implications. Curr Opin Pharmacol. 2001;1(6):583–90.
11. Furness JB. Types of neurons in the enteric nervous system. J Auton Nerv Syst. 2000; 81:87–96.
12. Shimizu Y, Matsuyama H, Shiina T, Takewaki T, Furness JB. Tachykinins and their functions in the gastrointestinal tract. Cell Mol Life Sci. 2008;65(2):295–311.
13. Maggi CA, Theodorsson E, Santicioli P, Patacchini R, Barbanti G, Turini D, et al. Motor response of the human isolated colon to capsaicin and its relationship to release of vasoactive intestinal polypeptide. Neuroscience. 1990;39(3):833–41.
14. Benko R, Lazar Z, Undi S, Illenyi L, Antal A, Horvath Ć-P, et al. Inhibition of nitric oxide synthesis blocks the inhibitory response to capsaicin in intestinal circular muscle preparations from different species. Life Sci. 2005;76(24):2773–82.
15. Gerard NP, Garraway LA, Eddy Jr RL, Shows TB, Iijima H, Paquet JL, et al. Human substance P receptor (NK-1): organization of the gene, chromosome localization, and functional expression of cDNA clones. Biochemistry. 1991;30(44):10640–6.
16. Gerard NP, Bao L, Xiao-Ping H, Gerard C. Molecular aspects of the tachykinin receptors. Regul Pept. 1993;43(1–2):21–35.
17. Regoli D, Drapeau G, Dion S, D'Orleans-Juste P. Receptors for substance P and related neurokinins. Pharmacology. 1989;38(1):1–15.
18. Regoli D, Boudon A, Fauchere JL. Receptors and antagonists for substance P and related peptides. Pharmacol Rev. 1994;46(4):551–99.
19. Kurtz MM, Wang R, Clements MK, Cascieri MA, Austin CP, Cunningham BR, et al. Identification, localization and receptor characterization of novel mammalian substance P-like peptides. Gene. 2002;296:205–12.
20. Boutaghou-Cherid H, Porcher C, Liberge M, Jule Y, Bunnett NW, Christen M-O. Expression of the neurokinin type 1 receptor in the human colon. Auton Neurosci. 2006;124(1): 9–17.
21. Jonsson M, Norrgard O, Forsgren S. Substance P and the neurokinin-1 receptor in relation to eosinophilia in ulcerative colitis. Peptides. 2005;26(5):799–814.
22. Bischoff SC, Schwengberg S, Lorentz A, Manns MP, Bektas H, Sann H, et al. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. Neurogastroenterol Motil. 2004;16(2):185–93.
23. Renzi D, Pellegrini B, Tonelli F, Surrenti C, Calabro A. Substance P (neurokinin-1) and neurokinin a (neurokinin-2) receptor gene and protein expression in the healthy and inflamed human intestine. Am J Pathol. 2000;157(5):1511–22.
24. Faussone-Pellegrini M-S. Relationships between neurokinin receptor-expressing interstitial cells of Cajal and tachykininergic nerves in the gut. J Cell Mol Med. 2006;10(1):20–32.
25. Kojima S-i, Ueda S, Ikeda M, Kamikawa Y. Calcitonin gene-related peptide facilitates serotonin release from guinea-pig colonic mucosa via myenteric neurons and tachykinin NK2/ NK3 receptors. Br J Pharmacol. 2004;141(3):385–90.
26. Douglas S, Leeman S. Neurokinin-1 receptor: functional significance in the immune system in reference to selected infections and inflammation. Ann N Y Acad Sci. 2011;1217: 83–95.
27. Fong TM, Anderson SA, Yu H, Huang RRC, Strader CD. Differential activation of intracellular effector by two isoforms of human neurokinin-1 receptor. Mol Pharmacol. 1992;41 (1):24–30. Date of Publication: 1992.; 1992.
28. Chernova I, Lai J-P, Li H, Schwartz L, Tuluc F, Korchak HM, et al. Substance P (SP) enhances CCL5-induced chemotaxis and intracellular signaling in human monocytes, which express the truncated neurokinin-1 receptor (NK1R). J Leukoc Biol. 2009;85(1):154–64.

29. Lai JP, Ho WZ, Kilpatrick LE, Wang X, Tuluc F, Korchak HM, et al. Full-length and truncated neurokinin-1 receptor expression and function during monocyte/macrophage differentiation. Proc Natl Acad Sci. 2006;103(20):7771–6.
30. Caberlotto L, Hurd YL, Murdock P, Wahlin JP, Melotto S, Corsi M, et al. Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain. Eur J Neurosci. 2003;17(9):1736–46.
31. Lai J-P, Lai S, Tuluc F, Tansky MF, Kilpatrick LE, Leeman SE, et al. Differences in the length of the carboxyl terminus mediate functional properties of neurokinin-1 receptor. Proc Natl Acad Sci U S A. 2008;105(34):12605–10.
32. Roush ED, Kwatra MM. Human substance P receptor expressed in Chinese hamster ovary cells directly activates G(alpha q/11), G(alpha s), G(alpha o). FEBS Lett. 1998;428(3): 291–4.
33. Macdonald SG, Dumas JJ, Boyd ND. Chemical cross-linking of the substance P (NK-1) receptor to the alpha subunits of the G proteins Gq and G11. Biochemistry. 1996;35 (9):2909–16.
34. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F, et al. The role of substance P in inflammatory disease. J Cell Physiol. 2004;201(2):167–80.
35. Khawaja AM, Rogers DF. Tachykinins: receptor to effector. Int J Biochem Cell Biol. 1996;28(7):721–38.
36. DeFea KA, Vaughn ZD, O'Bryan EM, Nishijima D, Dery O, Bunnett NW. The proliferative and antiapoptotic effects of substance P are facilitated by formation of a beta -arrestin-dependent scaffolding complex. Proc Natl Acad Sci U S A. 2000;97(20):11086–91.
37. Fiebich BL, Schleicher S, Butcher RD, Craig A, Lieb K. The neuropeptide substance P activates p38 mitogen-activated protein kinase resulting in IL-6 expression independently from NF-kappa B. J Immunol. 2000;165(10):5606–11.
38. Tokuda M, Miyamoto R, Sakuta T, Nagaoka S, Torii M, Tokuda M, et al. Substance P activates p38 mitogen-activated protein kinase to promote IL-6 induction in human dental pulp fibroblasts. Connect Tissue Res. 2005;46(3):153–8.
39. Lieb K, Fiebich BL, Berger M, Bauer J, Schulze-Osthoff K. The neuropeptide substance P activates transcription factor NF-kappa B and kappa B-dependent gene expression in human astrocytoma cells. J Immunol. 1997;159(10):4952–8.
40. Dumaz N, Marais R, Dumaz N, Marais R. Integrating signals between cAMP and the RAS/ RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft fur Biochemie und Molekularbiologie lecture delivered on 5 July 2003 at the special FEBS meeting in Brussels. FEBS J. 2005;272(14):3491–504.
41. Li H, Leeman SE, Slack BE, Hauser G, Saltsman WS, Krause JE, et al. A substance P (neurokinin-1) receptor mutant carboxyl-terminally truncated to resemble a naturally occurring receptor isoform displays enhanced responsiveness and resistance to desensitization. Proc Natl Acad Sci U S A. 1997;94(17):9475–80.
42. DeFea KA, Zalevsky J, Thoma MS, Dery O, Mullins RD, Bunnett NW. Beta-arrestin-dependent endocytosis of proteinase-activated receptor 2 is required for intracellular targeting of activated Erk1/2. J Cell Biol. 2000;148(6):1267–82.
43. Maggi CA, Giuliani S, Patacchini R, Santicioli P, Theodorsson E, Barbanti G, et al. Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Involvement of neurokinin-2 receptors. Gastroenterology. 1992;102:88–96.
44. Grider JR. Identification of neurotransmitters regulating intestinal peristaltic reflex in humans. Gastroenterology. 1989;97:1414–9.
45. Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V, Severini C, et al. The tachykinin peptide family. Pharmacol Rev. 2002;54(2):285–322.
46. Bartho L, Maggi CA, Wilhelm M, Patacchini R. Tachykinin NK1 and NK2 receptors mediate atropine-resistant ileal circular muscle contractions evoked by capsaicin. Eur J Pharmacol. 1994;259:187–93.
47. Harrison S, Geppetti P. Substance P. Int J Biochem Cell Biol. 2001;33(6):555–76.

48. Maggi CA, Patacchini R, Bartho L, Holzer P, Santicioli P. Tachykinin NK1 and NK2 receptor antagonists and atropine-resistant ascending excitatory reflex to the circular muscle of the guinea-pig ileum. Br J Pharmacol. 1994;112(1):161–8.
49. Maggi CA, Patacchini R, Meini S, Quartara L, Sisto A, Potier E, et al. Comparison of tachykinin NK1 and NK2 receptors in the circular muscle of the guinea-pig ileum and proximal colon. Br J Pharmacol. 1994;112(1):150–60.
50. D'antonio C, Wang B, McKay C, Huizinga JD. Substance P activates a non-selective cation channel in murine pacemaker ICC. Neurogastroenterol Motil. 2009;21(9):985–e79.
51. Kim BJ, Chang IY, Choi S, Jun JY, Jeon JH, Xu WX, et al. Involvement of Na+-leak channel in substance P-induced depolarization of pacemaking activity in interstitial cells of cajal. Cell Physiol Biochem. 2012;29(3–4):501–10.
52. Nakamura A, Tanaka T, Imanishi A, Kawamoto M, Toyoda M, Mizojiri G, et al. Bidirectional regulation of human colonic smooth muscle contractility by tachykinin NK2 receptors. J Pharmacol Sci. 2011;117(2):106–15.
53. Smith AS, Smid SD. Impaired capsaicin and neurokinin-evoked colonic motility in inflammatory bowel disease. J Gastroenterol Hepatol. 2005;20(5):697–704.
54. Kuwahara A, Cooke HJ. Tachykinin-induced anion secretion in guinea pig distal colon: role of neural and inflammatory mediators. J Pharmacol Exp Ther. 1990;252(1):1–7.
55. Carpenter DO, Briggs DB, Strominger N. Peptide-induced emesis in dogs. Behav Brain Res. 1984;11(3):277–81.
56. Horn CC. Why is the neurobiology of nausea and vomiting so important? Appetite. 2008;50:430–4.
57. Miller AD, Wilson VJ. "Vomiting center" reanalyzed: an electrical stimulation study. Brain Res. 1983;270(1):154–8.
58. Gardner CJ, Twissell DJ, Dale TJ, Gale JD, Jordan CC, Kilpatrick GJ, et al. The broad-spectrum anti-emetic activity of the novel non-peptide tachykinin NK1 receptor antagonist GR203040. Br J Pharmacol. 1995;116(8):3158–63.
59. Tattersall FD, Rycroft W, Hill RG, Hargreaves RJ. Enantioselective inhibition of apomorphine-induced emesis in the ferret by the neurokinin-1 receptor antagonist CP-99,994. Neuropharmacology. 1994;33(2):259–60.
60. Watson JW, Gonsalves SF, Fossa AA, McLean S, Seeger T, Obach S, et al. The antiemetic effects of CP-99,994 in the ferret and the dog – role of the NK1 receptor. Br J Pharmacol. 1995;115(1):84–94.
61. Furukawa N, Fukuda H, Hatano M, Koga T, Shiroshita Y. A neurokinin-1 receptor antagonist reduced hypersalivation and gastric contractility related to emesis in dogs. Am J Physiol Gastrointest Liver Physiol. 1998;275(5):G1193–201.
62. Campos D, Pereira JR, Reinhardt RR, Carracedo C, Poli S, Vogel C, et al. Prevention of cisplatin-induced emesis by the oral neurokinin-1 antagonist, MK-869, in combination with granisetron and dexamethasone or with dexamethasone alone. J Clin Oncol. 2001;19(6):1759–67.
63. Navari RM, Reinhardt RR, Gralla RJ, Kris MG, Hesketh PJ, Khojasteh A, et al. Reduction of cisplatin-induced emesis by a selective neurokinin-1Receptor antagonist. N Engl J Med. 1999;340(3):190–5.
64. Saito R, Takano Y, Kamiya H-o. Roles of substance P and NK1 receptor in the brainstem in the development of emesis. J Pharmacol Sci. 2003;91(2):87–94.
65. Edvinsson L, Rosendal-Helgesen S, Uddman R. Substance P: localization, concentration and release in cerebral arteries, choroid plexus and dura mater. Cell Tissue Res. 1983;234(1):1–7.
66. Steinman L. Elaborate interactions between the immune and nervous systems. Nat Immunol. 2004;5(6):575–81.
67. Vilisaar J, Kawabe K, Braitch M, Aram J, Furtun Y, Fahey A, et al. Reciprocal regulation of substance P and IL-12/IL-23 and the associated cytokines, IFNγ/IL-17: a perspective on the relevance of this interaction to multiple sclerosis. J Neuroimmune Pharmacol. 2015; 10(3):457–67.

68. Goode T, O'Connell J, Ho W-Z, O'Sullivan GC, Collins JK, Douglas SD, et al. Differential expression of neurokinin-1 receptor by human mucosal and peripheral lymphoid cells. Clin Diagn Lab Immunol. 2000;7(3):371–6.
69. Cooke HJ. Neuroimmune signaling in regulation of intestinal ion transport. Am J Physiol Gastrointest Liver Physiol. 1994;266(2):G167–78.
70. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science. 1988;241(4870):1218–21.
71. Laurenzi MA, Persson MA, Dalsgaard CJ, Haegerstrand A. The neuropeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells are preferentially influenced by the neuropeptide. Scand J Immunol. 1990;31(4):529–33.
72. Kincy-Cain T, Bost KL. Substance P-induced IL-12 production by murine macrophages. J Immunol. 1997;158(5):2334–9.
73. Ho WZ, Kaufman D, Uvaydova M, Douglas SD. Substance P augments interleukin-10 and tumor necrosis factor-alpha release by human cord blood monocytes and macrophages. J Neuroimmunol. 1996;71(1–2):73–80.
74. Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T cells. J Immunol. 1992;148(11):3498–504.
75. Blum AM, Metwali A, Cook G, Mathew RC, Elliott D, Weinstock JV. Substance P modulates antigen-induced, IFN-gamma production in murine Schistosomiasis mansoni. J Immunol. 1993;151(1):225–33.
76. Gitter BD, Regoli D, Howbert JJ, Glasebrook AL, Waters DC. Interleukin-6 secretion from human astrocytoma cells induced by substance P. J Neuroimmunol. 1994;51(1):101–8.
77. Martin FC, Anton PA, Gornbein JA, Shanahan F, Merrill JE. Production of interleukin-1 by microglia in response to substance P: role for a non-classical NK-1 receptor. J Neuroimmunol. 1993;42(1):53–60.
78. Martin FC, Charles AC, Sanderson MJ, Merrill JE. Substance P stimulates IL-1 production by astrocytes via intracellular calcium. Brain Res. 1992;599(1):13–8.
79. Lieb K, Schaller H, Bauer J, Berger M, Schulze-Osthoff K, Fiebich BL. Substance P and histamine induce interleukin-6 expression in human astrocytoma cells by a mechanism involving protein kinase C and nuclear factor-IL-6. J Neurochem. 1998;70(4):1577–83.
80. Blum AM, Metwali A, Crawford C, Li J, Qadir K, Elliott DE, et al. Interleukin 12 and antigen independently induce substance P receptor expression in T cells in murine schistosomiasis mansoni. FASEB J. 2001;15(6):950–7.
81. Arsenescu R, Blum AM, Metwali A, Elliott DE, Weinstock JV. IL-12 induction of mRNA encoding substance P in murine macrophages from the spleen and sites of inflammation. J Immunol. 2005;174(7):3906–11.
82. Weinstock J. Substance P, and the regulation of inflammation in infections and inflammatory bowel disease. Acta Physiol. 2014;2014(26):12428.
83. Raffa RB. Possible role(s) of neurokinins in CNS development and neurodegenerative or other disorders. Neurosci Biobehav Rev. 1998;22(6):789–813.
84. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol. 1983;131(4):1613–5.
85. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol. 1986;136(1):152–6.
86. Bost KL, Pascual DW. Substance P: a late-acting B lymphocyte differentiation cofactor. Am J Physiol. 1992;262(3 Pt 1):C537–45.
87. Wozniak A, Betts WH, McLennan G, Scicchitano R. Activation of human neutrophils by tachykinins: effect on formyl-methionyl-leucyl-phenylalanine- and platelet-activating factor-stimulated superoxide anion production and antibody-dependent cell-mediated cytotoxicity. Immunology. 1993;78(4):629–34.
88. Bar-Shavit Z, Goldman R, Stabinsky Y, Gottlieb P, Fridkin M, Teichberg VI, et al. Enhancement of phagocytosis – a newly found activity of substance P residing in its N-terminal tetrapeptide sequence. Biochem Biophys Res Commun. 1980;94(4):1445–51.

89. Haines KA, Kolasinski SL, Cronstein BN, Reibman J, Gold LI, Weissmann G. Chemoattraction of neutrophils by substance P and transforming growth factor-beta 1 is inadequately explained by current models of lipid remodeling. J Immunol. 1993;151(3):1491–9.
90. Ahluwalia A, De Felipe C, O'Brien J, Hunt SP, Perretti M. Impaired IL-1beta-induced neutrophil accumulation in tachykinin NK1 receptor knockout mice. Br J Pharmacol. 1998;124(6):1013–5.
91. Guo CJ, Lai JP, Luo HM, Douglas SD, Ho WZ, Guo CJ, et al. Substance P up-regulates macrophage inflammatory protein-1beta expression in human T lymphocytes. J Neuroimmunol. 2002;131(1–2):160–7.
92. Matis WL, Lavker RM, Murphy GF. Substance P induces the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium. J Invest Dermatol. 1990;94 (4):492–5.
93. Vishwanath R, Mukherjee R. Substance P promotes lymphocyte-endothelial cell adhesion preferentially via LFA-1/ICAM-1 interactions. J Neuroimmunol. 1996;71(1–2):163–71.
94. Weinstock JV, Blum A, Metwali A, Elliott D, Arsenescu R. IL-18 and IL-12 signal through the NF-kappa B pathway to induce NK-1R expression on T cells. J Immunol. 2003; 170(10):5003–7.
95. Blum A, Setiawan T, Hang L, Stoyanoff K, Weinstock JV. Interleukin-12 (IL-12) and IL-23 induction of substance P synthesis in murine T cells and macrophages is subject to IL-10 and transforming growth factor {beta} regulation. Infect Immun. 2008;76(8):3651–6.
96. Fiscus RR, Gross DR, Hao H, Wang X, Arden WA, Maley RH, et al. N omega-nitro-L-arginine blocks the second phase but not the first phase of the endothelium-dependent relaxations induced by substance P in isolated rings of pig carotid artery. J Cardiovasc Pharmacol. 1992;20 Suppl 12:S105–8.
97. Piedimonte G, Bertrand C, Geppetti P, Snider RM, Desai MC, Nadel JA. A new NK1 receptor antagonist (CP-99,994) prevents the increase in tracheal vascular permeability produced by hypertonic saline. J Pharmacol Exp Ther. 1993;266(1):270–3.
98. Nakanishi S. Substance P, precursor and kininogen: their structures, gene organizations, and regulation. Physiol Rev. 1987;67(4):1117–42.
99. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus D. Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. J Immunol. 1985;135(2):1331–7.
100. Khasabov SG, Rogers SD, Ghilardi JR, Peters CM, Mantyh PW, Simone DA. Spinal neurons that possess the substance P receptor are required for the development of central sensitization. J Neurosci. 2002;22(20):9086–98.
101. Lu C-L, Pasricha PJ, Hsieh J-C, Lu R-H, Lai C-R, Wu L-L, et al. Changes of the neuropeptides content and gene expression in spinal cord and dorsal root ganglion after noxious colorectal distension. Regul Pept. 2005;131:66–73.
102. Canning BJ, Spina D, Seybold VS. The role of peptides in central sensitization. Sensory nerves. In: Handbook of experimental pharmacology, vol. 194. Berlin/Heidelberg: Springer; 2009. p. 451–91.
103. Benson JR, Xu J, Moynes DM, Lapointe TK, Altier C, Vanner SJ, et al. Sustained neurochemical plasticity in central terminals of mouse DRG neurons following colitis. Cell Tissue Res. 2014;356(2):309–17.
104. Li WW, Guo TZ, Shi X, Sun Y, Wei T, Clark DJ, et al. Substance P spinal signaling induces glial activation and nociceptive sensitization after fracture. Neuroscience. 2015;310:73–90.
105. Weinstock J. Substance P, and the regulation of inflammation in infections and inflammatory bowel disease. Acta Physiol. 2015;213:453–61.
106. Beinborn M, Blum A, Hang L, Setiawan T, Schroeder J, Stoyanoff K, et al. TGF-beta regulates T-cell neurokinin-1 receptor internalization and function. Proc Natl Acad Sci U S A. 2010;107:4293–8.
107. Vasina V, Barbara G, Talamonti L, Stanghellini V, Corinaldesi R, Tonini M, et al. Enteric neuroplasticity evoked by inflammation. Auton Neurosci. 2006;126:264–72.
108. Mawe GM, Strong DS, Sharkey KA. Plasticity of enteric nerve functions in the inflamed and postinflamed gut. Neurogastroenterol Motil. 2009;21(5):481–91.

109. von Boyen GBT, Reinshagen M, Steinkamp M, Adler G, Kirsch J. Enteric nervous plasticity and development: dependence on neurotrophic factors. J Gastroenterol. 2002;37(8):583–8.
110. Goode T, O'Connell J, Anton P, Wong H, Reeve J, O'Sullivan G, et al. Neurokinin-1 receptor expression in inflammatory bowel disease: molecular quantitation and localisation. Gut. 2000;47(3):387–96.
111. Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EP, Vigna SR, et al. Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn disease. Proc Natl Acad Sci U S A. 1988;85(9):3235–9.
112. Goode T, O'Connor T, Hopkins A, Moriarty D, O'Sullivan GC, Collins JK, et al. Neurokinin-1 receptor (NK-1R) expression is induced in human colonic epithelial cells by proinflammatory cytokines and mediates proliferation in response to substance P. J Cell Physiol. 2003; 197(1):30–41.
113. Sideri A, Bakirtzi K, Shih DQ, Koon HW, Fleshner P, Arsenescu R, et al. Substance P mediates pro-inflammatory cytokine release form mesenteric adipocytes in inflammatory bowel disease patients. Cell Mol Gastroenterol Hepatol. 2015;1:420–32.
114. Koch TR, Carney JA, Go VL. Distribution and quantitation of gut neuropeptides in normal intestine and inflammatory bowel diseases. Dig Dis Sci. 1987;32:369–76.
115. Mazumdar S, Das KM. Immunocytochemical localization of vasoactive intestinal peptide and substance P in the colon from normal subjects and patients with inflammatory bowel disease. Am J Gastroenterol. 1992;87:176–81.
116. Bernstein CN, Robert ME, Eysselein VE. Rectal substance P concentrations are increased in ulcerative colitis but not in Crohn's disease. Am J Gastroenterol. 1993;88:908–13.
117. Kimura M, Masuda T, Hiwatashi N, Toyota T, Nagura H. Changes in neuropeptide-containing nerves in human colonic mucosa with inflammatory bowel disease. Pathol Int. 1994;44:624–34.
118. Weinstock JV, Blum A, Metwali A, Elliott D, Bunnett N, Arsenescu R. Substance P regulates Th1-type colitis in IL-10 knockout mice. J Immunol. 2003;171(7):3762–7.
119. Jacoby DB, Yost BL, Elwood T, Fryer AD. Effects of neurokinin receptor antagonists in virus-infected airways. Am J Physiol Lung Cell Mol Physiol. 2000;279(1):L59–65.
120. Tripp RA, Moore D, Winter J, Anderson LJ. Respiratory syncytial virus infection and G and/or SH protein expression contribute to substance P, which mediates inflammation and enhanced pulmonary disease in BALB/c mice. J Virol. 2000;74(4):1614–22.
121. Kwan ML, Gomez AD, Baluk P, Hashizume H, McDonald DM. Airway vasculature after mycoplasma infection: chronic leakiness and selective hypersensitivity to substance P. Am J Physiol Lung Cell Mol Physiol. 2001;280(2):L286–97.
122. Blum A, Metwali A, Elliott D, Weinstock J. T cell substance P receptor governs antigen-elicited IFN-gamma production. Am J Physiol Gastrointest Liver Physiol. 2003;284(2): G197–204.
123. Blum AM, Metwali A, Kim-Miller M, Li J, Qadir K, Elliott DE, et al. The substance P receptor is necessary for a normal granulomatous response in murine schistosomiasis mansoni. J Immunol. 1999;162(10):6080–5.
124. Blum AM, Elliott DE, Metwali A, Li J, Qadir K, Weinstock JV. Substance P regulates somatostatin expression in inflammation. J Immunol. 1998;161(11):6316–22.
125. Kincy-Cain T, Bost KL. Increased susceptibility of mice to Salmonella infection following in vivo treatment with the substance P antagonist, spantide II. J Immunol. 1996;157(1): 255–64.
126. Svensson A, Kaim J, Mallard C, Olsson A, Brodin E, Hokfelt T, et al. Neurokinin 1 receptor signaling affects the local innate immune defense against genital herpes virus infection. J Immunol. 2005;175(10):6802–11.
127. Castagliuolo I, Riegler M, Pasha A, Nikulasson S, Lu B, Gerard C, et al. Neurokinin-1 (NK-1) receptor is required in Clostridium difficile- induced enteritis. J Clin Invest. 1998;101(8): 1547–50.

128. Pothoulakis C, Castagliuolo I, LaMont JT, Jaffer A, O'Keane JC, Snider RM, et al. CP-96,345, a substance P antagonist, inhibits rat intestinal responses to Clostridium difficile toxin A but not cholera toxin. Proc Natl Acad Sci U S A. 1994;91(3):947–51.
129. Puneet P, Hegde A, Ng SW, Lau HY, Lu J, Moochhala SM, et al. Preprotachykinin-a gene products are key mediators of lung injury in polymicrobial sepsis. J Immunol. 2006;176(6): 3813–20.
130. Hegde A, Zhang H, Moochhala SM, Bhatia M. Neurokinin-1 receptor antagonist treatment protects mice against lung injury in polymicrobial sepsis. J Leukoc Biol. 2007;82(3): 678–85.
131. Ng SW, Zhang H, Hegde A, Bhatia M. Role of preprotachykinin-A gene products on multiple organ injury in LPS-induced endotoxemia. J Leukoc Biol. 2008;83(2):288–95.
132. Hegde A, Tamizhselvi R, Manikandan J, Melendez AJ, Moochhala SM, Bhatia M. Substance P in polymicrobial sepsis: molecular fingerprint of lung injury in preprotachykinin-a-/- mice. Mol Med. 2009;16(5–6):188–98.
133. Lai JP, Ho WZ, Zhan GX, Yi Y, Collman RG, Douglas SD. Substance P antagonist (CP-96,345) inhibits HIV-1 replication in human mononuclear phagocytes. Proc Natl Acad Sci U S A. 2001;98(7):3970–5.
134. Ho W-Z, Lai J-P, Li Y, Douglas SD. HIV enhances substance P expression in human immune cells. FASEB J. 2002;16:616–8.
135. McGowan IM, Fairhurst RM, Shanahan F, Anton PA. Mucosal substance P receptor expression in HIV infection and inflammatory bowel disease. Neuroimmunomodulation. 1997;4:70–6.
136. Patel L, Lindley C. Aprepitant--a novel NK1-receptor antagonist. Expert Opin Pharmacother. 2003;4(12):2279–96.
137. Navari RM. Profile of netupitant/palonosetron (NEPA) fixed dose combination and its potential in the treatment of chemotherapy-induced nausea and vomiting (CINV). Drug Des Devel Ther. 2015;9:155–61.
138. Tebas P, Spitsin S, Barrett JS, Tuluc F, Elci O, Korelitz JJ, et al. Reduction of soluble CD163, substance P, programmed death 1 and inflammatory markers: phase 1B trial of aprepitant in HIV-1-infected adults. AIDS (London, England). 2015;29(8):931–9.
139. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219–42.

Chapter 5
Immunomodulation by Vasoactive Intestinal Polypeptide (VIP)

Kirsten M. Hooper, Weimin Kong, and Doina Ganea

Abstract Vasoactive intestinal peptide (VIP) is one of the major neuropeptides expressed and produced by the enteric nervous system. VIP release in the proximity of GI resident immune cells facilitates its immunoregulatory functions, including effects on macrophages, dendritic cells, and T lymphocytes. Here we discuss the functions of VIP as a modulator and possible therapeutic target in gastrointestinal inflammation in the larger context of its general immunoregulatory role.

Keywords Vasoactive intestinal peptide • T cell differentiation • Tolerogenic dendritic cells • Inflammatory bowel disease • Immunoregulation • Neuropeptides

Abbreviations

APC	Antigen-presenting cell
CD	Crohn's disease
CIA	Collagen-induced arthritis
CLP	Cecal ligation and puncture
CNS	Central nervous system
DC	Dendritic cell
DSS	Dextran sodium sulfate
EAE	Experimental autoimmune encephalomyelitis
ENS	Enteric nervous system
GI Tract	Gastrointestinal tract
HMGB1	High mobility group B1
IBD	Inflammatory bowel disease
LN	Lymph node
MS	Multiple sclerosis
RA	Retinoic acid

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tDC	Tolerogenic dendritic cells
TLR	Toll-like receptor
TNBS	Trinitrobenzene sulfonic acid
UC	Ulcerative colitis
VIP	Vasoactive intestinal peptide

Introduction

The autonomic enteric nervous system (ENS) provides a major venue of communication between the central nervous system (CNS) and the gastrointestinal (GI) tract. The ENS is organized into myenteric and submucosal plexi and consists of various types of neurons, glial cells, and sympathetic and parasympathetic nerve fibers. The close proximity to immune cells allows direct immunomodulatory effects of neuronal-derived neurotransmitters and neuropeptides on immune cells expressing the appropriate receptors. Immunomodulatory neuropeptides expressed and released by the ENS include vasoactive intestinal peptide (VIP), substance P, calcitonin gene-related peptide, and neuropeptide Y [1, 2].

VIP is one of the gut's most abundant neuropeptides, present at high concentrations in the myenteric plexus [3]. VIPergic enteric neurons project to muscle and crypt epithelial cells [4] controlling intestinal motility, water, and electrolyte secretion [5–10]. The role of VIP in intestinal development and function is supported by the fact that VIP-deficient mice exhibit intestinal anomalies such as longer villi, thicker smooth muscle layers, increased numbers of goblet cells deficient in mucus production, and impaired intestinal transit [11]. In addition, due to its immunoregulatory activities, VIP emerged recently as a possible therapeutic target in inflammatory bowel diseases (IBD) [1]. In this chapter, we discuss VIP as a modulator and possible therapeutic target in gastrointestinal inflammation, in the larger context of its well-described general immunoregulatory role.

VIP: Structure and Synthesis

VIP, a 28-amino acid peptide, belongs to the secretin/glucagon family, sharing the most homology (68 %) with another neuropeptide, i.e., pituitary adenylate cyclase-activating polypeptide (PACAP). VIP and PACAP are presumed to have resulted from the duplication of a common ancestral gene [12, 13]. Mature VIP is processed from a pre-pro-protein which is also the source of a second peptide called peptide histidine methionine (PHM) in humans and peptide histidine isoleucine (PHI) in other mammals. With the exception of guinea pig, the amino acid composition of VIP is identical in all mammals, showing remarkable conservation during evolution [14, 15].

VIP is present in most organs [16] including the GI tract and the immune system where it is released from both neuronal and nonneuronal sources. The fact that autonomic denervation does not affect VIP levels in thymus and spleen [17] is an indication that nonneuronal cells represent the major VIP source in primary and secondary lymphoid organs. Although numerous cell types such as basophils, mast cells, and neutrophils produce VIP variants (reviewed in [15]), T lymphocytes represent a major VIP source within lymphoid organs and tissues (reviewed in [18]). Indeed, both CD4 and CD8 T cells were reported to express VIP mRNA and to process the VIP pre-proprotein [19], and antigen-activated Th2 CD4 and T2 CD8 T cells were shown to secrete significant amounts of mature VIP [20]. The role of endogenous immune VIP has been recently addressed. Elimination of endogenous T cell-derived VIP in purified CD4 T cell cultures through the use of VIPase resulted in a predominant Th1 phenotype, supporting the role of immune VIP in promoting Th2 differentiation and/or survival [21]. In vivo, higher numbers of anti-viral CD8 T cells were generated in wild-type bone marrow chimeras engrafted with VIP-deficient hematopoietic cells, supporting the anti-inflammatory role of endogenous immune VIP [22]. Since the GI tract is rich in lymphocytes, immune VIP could be a significant player during inflammatory conditions. However, for the time being, the presence and role of immune VIP in intestinal physiology and inflammation remains to be ascertained.

VIP levels are altered in several pathological conditions. Reduced levels of VIP were reported in the cerebral spinal fluid of multiple sclerosis (MS) patients and in serum of patients with rheumatoid arthritis and Sjogren's syndrome [23–25]. Also, high levels of VIPase autoantibodies and low concentrations of VIP were reported in the serum of lupus and autoimmune thyroiditis patients [26]. Conflicting results were reported in terms of changes in VIP levels in human IBD. A number of studies reported an increase in VIP immunoreactivity in mucosal and submucosal colon and in rectal biopsies from patients with Crohn's disease (CD), but not ulcerative colitis (UC) [27–29], whereas others reported a decrease in VIP immunoreactivity in both CD and UC intestinal samples [30, 31]. In the TNBS colitis model, Baticic et al. [32] reported increased VIP levels in serum, colon, and brain, whereas Sigalet et al. [33] reported a decrease in the number of VIPergic enteric neurons. These discrepancies could reflect differences in the location of the sampled areas and/or in lesion severity. Indeed, it has been proposed that areas of high inflammation lose neuropeptide innervation, whereas increased neuropeptide expression occurs in nearby tissue and regenerating areas [34]. For the moment, questions whether IBD is associated with increased or decreased VIP levels and whether such changes correlate with disease severity remain to be addressed.

VIP Receptors

Three types of VIP/PACAP receptors, i.e., VPAC1, VPAC2, and PAC1, are widely distributed in CNS and peripheral organs, including immune cells and organs [35–37]. VPAC1 and VPAC2 bind VIP and PACAP with equal high affinity, whereas

PAC1 is a PACAP-preferring receptor. Both VPAC1 and VPAC2 are class B G-protein-coupled receptors signaling primarily, but not exclusively, through activation of adenylate cyclase (reviewed in [37, 38]). Immune cells such as lymphocytes, macrophages, dendritic cells, microglia, monocytes, and mast cells express VPAC1 constitutively, and VPAC2 expression, although low in resting cells, is upregulated following activation, particularly in T cells (reviewed in [39]).

Although VPAC1 was characterized as the main mediator of VIP effects on innate immune cells, further studies identified VPAC2 as the major signaling receptor for the effects on CD4 T cell differentiation (reviewed in [39–41]). Changes in VIP receptor expression have been reported in several autoimmune/inflammatory diseases. Immune cells from patients with ankylosing spondylitis and osteoarthritis express lower VPAC1 levels and respond poorly to VIP, and in patients with rheumatoid arthritis, lower VPAC1 levels are associated with a polymorphism in the 3'UTR of the VPAC1 gene [42–45]. Reduced VPAC2 expression associated with a distinct DNA footprinting pattern in the VPAC2 promoter was reported in MS patients [45], whereas monocytes from Sjogren's patients were shown to express higher VPAC2 levels associated with deficiencies in the phagocytosis of apoptotic cells [46].

The physiological role of VPAC1 and VPAC2 in the intestine has been addressed in studies related to electrolyte secretion and muscle contractility. VPAC2 receptors on smooth muscle mediate the relaxation of the circular muscle, and both VPAC2 and VPAC1 were reported to mediate the stimulatory effect of VIP on neurogenic contractions of the longitudinal muscle [7–9, 47]. In addition, epithelial VPAC1 is involved in VIP-induced electrolyte secretion [7]. In contrast, the role of VIP receptors in intestinal immune cells is not elucidated. Interestingly, both receptors are expressed in Peyer's patch dendritic cells (PP-DC) at higher levels than in DC residing outside the GI tract [48]. It is tempting to speculate that this might contribute to homeostasis in the GI tract through VIP-induced maintenance of a tolerogenic phenotype in DC. This is supported by the previously reported capacity of VIP to induce IL-10-producing tolerogenic DC [49, 50]. Whether IBD is associated with changes in VPAC1 and VPAC2 expression remains to be determined. Presently, there is only one report from Yukawa and colleagues [51] reporting increased VPAC1 expression in lamina propria CD3+ T cells and $CD3^{-}CD19^{-}CD68^{+}$ macrophage-type mucosal cells in UC patients and to a lesser degree in CD patients.

VIP: Immunoregulatory Activity in Innate and Adaptive Immunity (Fig. 5.1)

Innate Effector Immune Cells

Monocytes, macrophages, dendritic cells, and microglia express VIP receptors, primarily VPAC1 [52–54]. Exogenous VIP acts as an anti-inflammatory agent inhibiting expression of iNOS and of proinflammatory cytokines and chemokines and

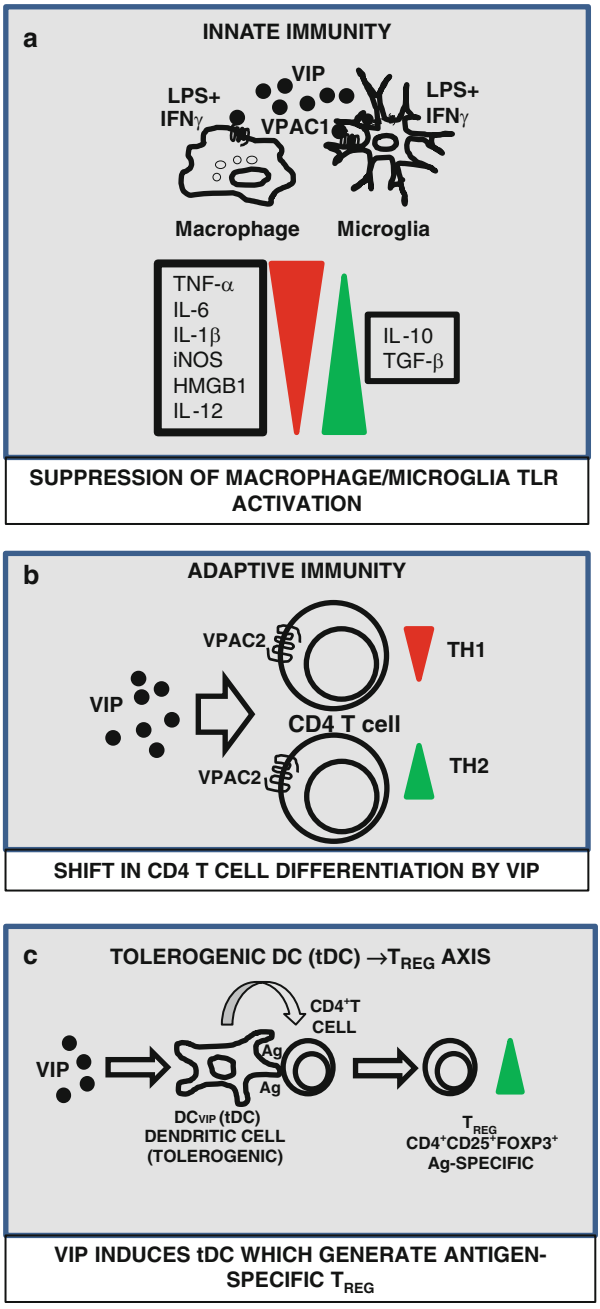

Fig. 5.1 Effects of VIP in innate and adaptive immunity. VIP suppresses activation of innate immune cells (macrophages, monocytes, and microglia) in response to TLR signaling. VIP inhibits proinflammatory factors and promotes the expression and release of anti-inflammatory cytokines through VPAC1 (**a**). VIP affects CD4 T cell differentiation shifting the Th1/Th2 balance in favor of Th2 through VPAC2 (**b**). Generation of DC in the presence of VIP (DC_{VIP}) leads to tolerogenic DC (tDC) which induce $CD4^+CD25^+Foxp3^+$ Treg specific for the antigen presented by DC_{VIP} (**c**)

stimulating production of IL-10 in TLR-stimulated monocytes, macrophages, and microglia in vitro and in vivo (reviewed in [39]). VIP also downregulates the release of HMGB1, a late-occurring cytokine critical in endotoxemia and sepsis [55]. In addition, VIP reduces TLR2 and TLR4 expression in innate immune cells in a model of experimental colitis and in human rheumatoid synovial fibroblasts [56–59].

The immunosuppressive effects of VIP are mediated primarily through VPAC1 and involve the regulation of several transcription factors, i.e., AP-1, NFkB, CREB, and IRF-1 (Fig. 5.2) (reviewed in [18, 39]). The inhibition of AP-1 and especially NFkB, transcription factors essential for the expression of proinflammatory genes, results in effects on a wide range of cytokines/chemokines. The cAMP-independent pathway activated by VIP stabilizes IkB and maintains the p65/p50/IkB complex in the cytoplasm. The cAMP-dependent pathway contributes to nuclear translocation of phosphorylated CREB and to the sequestration of the coactivator CBP. As a result, NFkB transcriptional activity is impaired [60]. The cAMP-dependent pathway also inhibits STAT1 phosphorylation and reduces IRF-1 binding to iNOS and IL-12p40 promoters [61]. In addition, the cAMP-dependent pathway inhibits the MEKK1/MEK4/JNK pathway and c-Jun phosphorylation and induces JunB synthesis, changing AP-1 composition and binding to the TNFα promoter [62]. Moreover, through effects on MEKK1/MEK3/6/p38, VIP inhibits TATA-box-binding protein (TBP) phosphorylation reducing RNA pol II recruitment [63].

Tolerogenic Dendritic Cells (tDC)

In addition to its inhibitory effect on activated innate immune cells, the immunosuppressive activity of VIP is also mediated through its capacity to generate tolerogenic DC (tDC). In steady-state conditions, DC carrying self-antigens contribute to tolerance. Therefore, the generation of antigen-specific tDC represents a major therapeutic target in the treatment of autoimmune/inflammatory diseases. A number of biological agents including galectin 1, vitamin D3, IL-10, TNFα, and more recently VIP were reported to generate tDC which could be manipulated to present specific autoantigens [64]. In vitro exposure to VIP during DC differentiation resulted in the induction of IL-10-producing tolerogenic DC (DC_{VIP}) capable of inducing antigen-specific $CD4^+Foxp3^+$ Treg in vivo and in vitro [49, 50]. In experimental models, inoculation of antigen-pulsed DC_{VIP} resulted in the generation of Treg specific for the antigen carried by DC_{VIP}. The Treg exhibited reduced proliferation, reduced IL-2 and IFNγ production, and increased Foxp3 and IL-10 expression [50]. In a model of collagen-induced arthritis (CIA), DC_{VIP} pulsed with collagen II stopped disease progression and reduced T cell proliferation and IFNγ production. This was an antigen-specific event, since DC_{VIP} pulsed with OVA did not affect arthritis, although they did inhibit OVA-induced delayed-type hypersensitivity [65]. In a bone marrow transplantation model, DC_{VIP}-generated Treg prevented graft-versus-host disease but maintained the graft-versus-tumor response [66]. More recently, DC transduced with lentiviral vectors expressing VIP were shown to be therapeutic in experimental

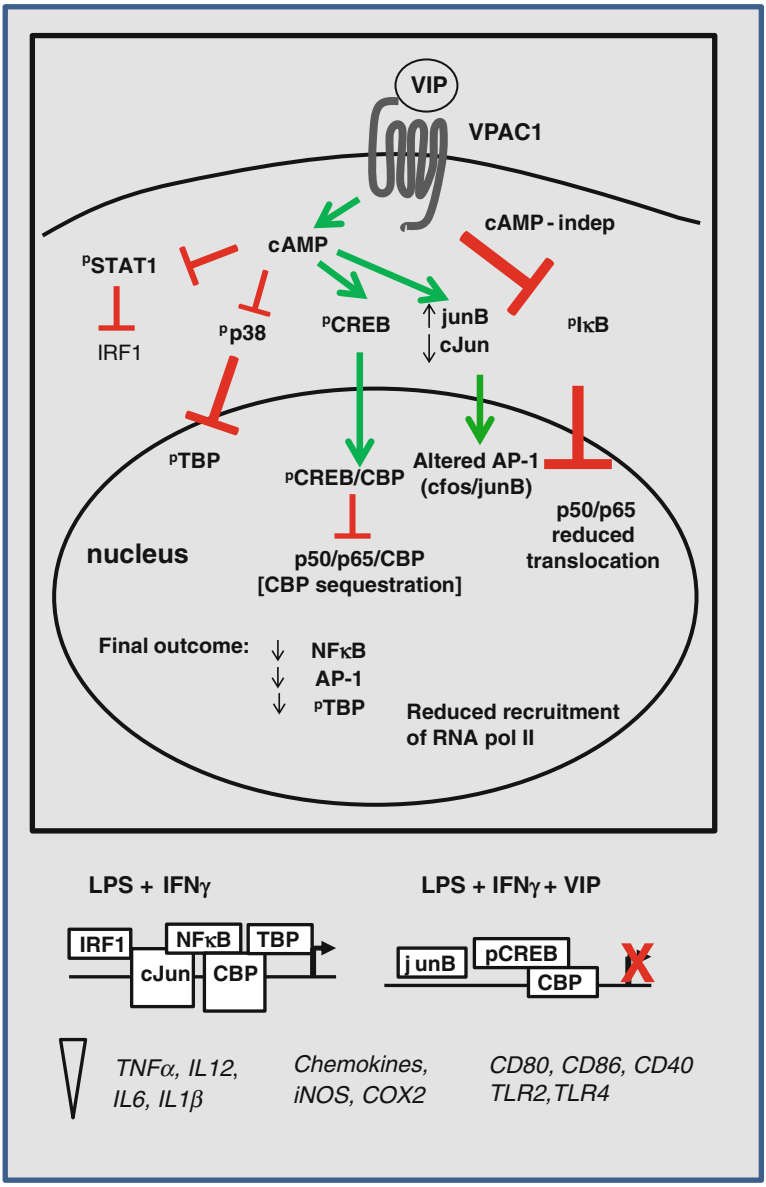

Fig. 5.2 Signaling pathways involved in VIP suppression of macrophage/monocyte innate immune responses. VIP binding to VPAC1 initiates activation of adenylate cyclase resulting in cAMP increases which affect several downstream signaling molecules: inhibition of STAT1 phosphorylation and IRF1 induction; inhibition of p38 MAPK phosphorylation and downstream phosphorylation of TATA-box-binding protein (TBP); increased phosphorylation of CREB followed by increased nuclear translocation; inhibition of JNK resulting in reduced c-Jun expression; upregulation of JunB expression leading to changes in AP-1 composition. A second, cAMP-independent signaling pathway results in stabilization of cytoplasmic NFkB complexes by inhibiting IkB phosphorylation and degradation. Reduced NFkB transactivation following VIP binding is due to both lower levels of nuclear p50/p65 complexes and to lack of CBP due to its sequestration by PCREB. Ultimate consequences are the reduced expression of a variety of proinflammatory cytokines, chemokines, iNOS, as well as of TLRs and costimulatory molecule (CD80, CD86, and CD40) expression

autoimmune encephalomyelitis (EAE) and sepsis models [67]. Due to their capacity to migrate to inflammatory sites, VIP-expressing DC are expected to deliver anti-inflammatory VIP locally at the inflammation site, in addition to inducing antigen-specific Treg. Therefore, the development of VIP-expressing DC generated from human blood monocytes, loaded with relevant autoantigens, and reinjected into the patient represents an enticing prospect in the treatment of autoimmune diseases.

In the intestine, distinct DC subpopulations were shown to generate an active adaptive immune response against pathogens and to induce and maintain tolerance to food antigens and commensal bacteria (reviewed in [68–70]). The major distinction between these subsets resides in the expression of the mutually exclusive CD103 and CX3CR1 markers. The nonmigratory inflammatory $CD103^{-}CX3CR1^{+}$ subset found primarily in the lamina propria is of macrophage lineage. In addition, a recently identified DC-lineage $CD103^{-}CX3CR1^{int}CCR2^{+}$ subpopulation migrates to mesenteric lymph nodes (MLN) and drives IL-17 production in T cells [71].

In contrast, $CD103^{+}CX3CR1^{-}$ tolerogenic DC in the lamina propria acquire food and self-antigens from $CX3CR1^{+}$ macrophages and migrate to MLN where they induce antigen-specific $CCR9^{+}\alpha4\beta7^{+}$ Treg through secretion of retinoic acid and TGFβ [72, 73]. Based on its abundant presence in the lamina propria, and its well-described DC tolerogenic activity, VIP has been proposed to be involved in the induction and maintenance of $CD103^{+}$ tolerogenic DC [70]. In addition, VIP could play an important role in reducing intestinal inflammation through its inhibitory effects on proinflammatory cytokine production by macrophages and its shift during T cell differentiation toward Th2/Treg, at the expense of Th1/Th17 effectors (Fig. 5.3).

Adaptive Immunity: Activation and Differentiation of CD4 T Lymphocytes

Th1 Versus Th2 Cells

Activation of naïve CD4 T cells involves interactions with DC and delivery of both stimulatory and costimulatory signals. VIP reduces DC capacity to deliver the costimulatory signals by preventing the upregulation of CD40, CD80, and CD86, resulting in reduced T cell proliferation [74, 75]. In addition, VIP also affects CD4 T cell differentiation by preferentially promoting in vivo and in vitro Th2 differentiation at the expense of the proinflammatory Th1 subset (reviewed in [18, 76]). The VIP-induced Th2 bias is mediated indirectly through inhibition of IL-12 production by activated antigen-presenting cells (APCs) and directly by blocking IL-12 signaling and inducing c-Maf and JunB in T cells [77, 78]. In addition, VIP also supports Th2 survival in vivo and in vitro by inhibiting FasL and granzyme B expression in Th2 cells [79, 80]. The role of endogenous VIP and of the VPAC2 receptor in promoting Th2 responses in vivo was demonstrated by comparing T cell responses to specific antigens in VPAC2 KO and transgenic mice overexpressing the human VPAC2 receptor [81, 82].

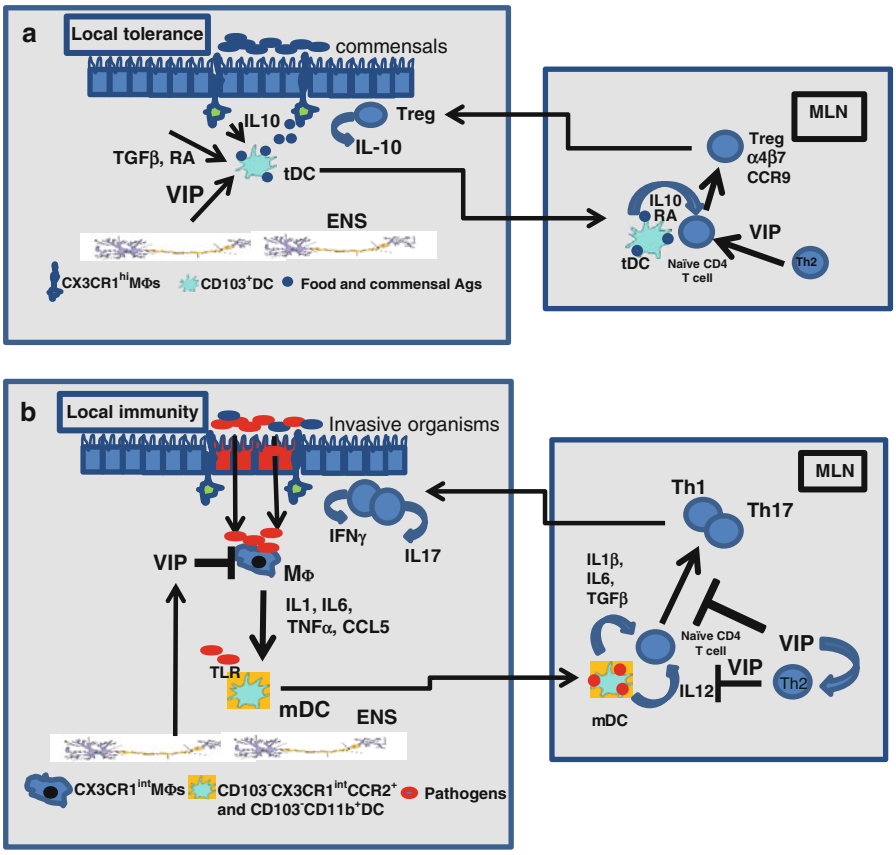

Fig. 5.3 Model for the anti-inflammatory role of VIP in the intestinal microenvironment. In the absence of pathogens, commensals and food proteins are taken in by LP CD103$^+$ DC tolerized by a combination of IL-10 (produced by CX3CR1^{hi} regulatory macrophages), TGFβ, RA (produced by epithelial cells), and VIP (produced by ENS). tDC migrate to MLN where they generate antigen-specific Treg through secretion of IL10 and RA. VIP produced by immune cells in MLN contributes to T cell differentiation into the Th2/Treg phenotype. tDC induction of α4β7 and CCR9 on the newly generated Treg enables them to home to the intestine where they induce and maintain local tolerance (**a**). Invasive pathogens are taken in by CX3CR1^{int} macrophages differentiated from Ly6C$^+$CCR2$^+$ monocytes and transferred to LP DC. Following TLR stimulation by pathogens, DC mature into proinflammatory APCs and migrate to MLN where they activate T cells to differentiate into Th1 and Th17 subsets. The proinflammatory Th1/Th17 cells migrate back to the intestine and initiate the adaptive proinflammatory response. VIP can still exert a suppressive effect by (**a**) inhibiting macrophage activation and reducing costimulatory molecule expression during DC maturation in LA and (**b**) by inhibiting the production of proinflammatory cytokines involved in T cell differentiation and shifting the Th1/Th2 balance in favor of Th2 in MLN (**b**). *ENS* enteric nervous system, *LP* lamina propria, *MLN* mesenteric lymph nodes, *RA* retinoic acid, *tDC* tolerogenic dendritic cells, *TLR* Toll-like receptors

Th17 Cells

Proinflammatory Th17 cells are major players in autoimmune/inflammatory diseases such as rheumatoid arthritis, MS, psoriasis, and CD [83–86]. The effect of VIP on Th17 differentiation is still under debate. In models of type I diabetes and collagen-induced arthritis (CIA), VIP administration resulted in delayed disease onset, reduced levels of IL-17, lower percentage of splenic IL-17$^+$ T cells, and reduced expression of STAT3 and RORγt [87, 88]. However, exposure to VIP during in vitro differentiation of Th17 cells resulted in higher numbers of IL-17$^+$ T cells [89–91]. Further studies are required to determine whether VIP induces Th17 in vivo and whether VIP-induced cells express the pathogenic Th17 signature [92].

Regulatory T Cells (Treg)

Treg play an essential role in maintaining tolerance and controlling the extent of an ongoing immune response. Deficiencies in Treg were reported in experimental models and in human autoimmune diseases. In vivo administration of VIP together with low doses of antigen led to increased numbers of functional CD4$^+$CD25$^+$Foxp3$^+$ Treg which inhibited antigen-specific T cell proliferation, reduced Th1 responses, and transferred suppression to naïve recipients [49]. The nature of the VIP-induced Treg and whether VIP affected T cells directly or through APCs remains to be established. In humans, the use of nebulized VIP in patients with sarcoidosis also resulted in increased numbers of CD4$^+$ CD25$^+$ Foxp3$^+$ Treg in the bronchoalveolar fluid [93]. More relevant to autoimmune conditions, VIP administration in CIA, murine type I diabetes, and EAE resulted in the generation of Treg and a decrease in Th17 [87, 88, 94, 95]. Moreover, Treg from VIP-treated arthritic mice were able to ameliorate disease progression when transferred to mice with established disease [96]. Significant disease amelioration associated with reduction in inflammation and induction of CD4$^+$CD25$^+$Foxp3$^+$ Treg also occurred upon in vivo delivery of a VIP-expressing lentiviral vector to arthritic mice [97]. However, although these studies were proof of concept that VIP induced functional Treg in vivo, the therapeutic use of VIP in patients would require improved methods of targeted delivery combined with protection against peptide degradation.

VIP Involvement in Sepsis and IBD

A large body of literature supports the concept that VIP could be part of a feedback circuit which limits ongoing inflammatory responses in the CNS and peripheral organs. Treatment with exogenous VIP at onset or in established disease proved beneficial in a variety of experimental models, such as CIA, EAE, type I diabetes, uveoretinitis, pancreatitis, and chronic obstructive pulmonary disease (COPD), as well as

Table 5.1 VIP/VIP-R genetic models

Genetic model	Disease model	Effect	References
VIP KO	EAE	Significant resistance to clinical disease Increased numbers of encephalitogenic Th1/Th17 capable of transferring EAE T cells do not infiltrate CNS parenchyma	[128]
	LPS endotoxemia	Significant resistance to clinical disease Decreased serum levels of proinflammatory cytokines Impaired NFkB activation in peritoneal cells	[111]
	DSS colitis	Resistance to clinical disease Reduced colonic expression of IL-6 and TNFα	[121]
	TNBS colitis	Milder clinical profile Hyperactivation of splenic T cell response Lower serum and colonic TNFα and IL-6 levels	[119]
PACAP KO	EAE	Exacerbated disease Increased Th1/Th17 Decreased Th2/Treg	[133, 134]
	DSS colitis	Exacerbated disease Colorectal tumors with aggressive pathology	[132]
VPAC1 KO	DSS colitis	Attenuated clinical disease	[126]
VPAC2 KO	DSS colitis	Exacerbated disease Higher levels of IL-6, IL-1β, and MMP-9	[126]
	EAE	Exacerbated disease Higher levels of TNFα, IL-6, IFNγ, IL-17 in CNS and LNs Lower levels of IL-10, IL-4, Foxp3 in CNS, thymus, LNs	[122]
	DTH	Exacerbated clinical disease	[81]
VPAC2 Tg (T cells)	DTH	Reduced clinical disease	[81]
	Cutaneous allergic reaction	Exacerbated clinical symptoms	[82]
PAC1 KO	LPS endotoxemia	Exacerbated disease	[109]

in models of neurodegeneration or CNS trauma such as Parkinson disease, traumatic brain injury, and spinal cord injury (reviewed in [98]). Here we review studies related to VIP in models of sepsis and IBD, using exogenous VIP administration and VIP/VIP receptor genetic models (Table 5.1).

Sepsis

Sepsis refers to a severe and often fatal systemic inflammatory response initiated by pathogens. The often observed cardiovascular collapse and progressive multiorgan failure are caused by high systemic concentrations of proinflammatory immune

mediators, both early and late cytokines (TNFα, IL-1β, IL-6, and HMGB1, respectively), as well as high expression of iNOS and of cellular tissue factor (TF) [99–103]. Although a marked discrepancy in LPS sensitivity exists between mice and humans [104], mice are the most common animal models for sepsis, i.e., inoculation of high LPS doses (endotoxemic shock) and cecal ligation and puncture (CLP). An improved system recently developed uses CLP in mice with human lineage immune cells and should provide clinically relevant information [105].

Positive correlations have been established between serum VIP concentrations and endotoxemic shock in mice, dogs, and pigs injected with LPS, as well as in human patients with meningococcal septicemia (reviewed in [106]). Beneficial effects of VIP administration were reported for both LPS-induced septic shock and CLP and shown to be associated with decreases in serum IL-6, TNFα, IL-12, HMGB1, and NO and increases in IL-10 [55, 107, 108]. All three receptors, VPAC1, VPAC2, and PAC1, contribute to various degrees of the protective effect of VIP and PACAP in LPS-induced septic shock [107, 109, 110]. More recently, i.p. administration of lentiVIP-DC (VIP-secreting tolerogenic DC) in the CLP model showed significant protection associated with decreased proinflammatory cytokines and increased IL-10 in the peritoneal fluid [67].

Based on the protective effects of exogenous VIP, lack of endogenous VIP was expected to heighten susceptibility to septic shock. However, contrary to expectations, global VIP-deficient mice exhibited significant resistance to LPS administration (Table 5.1), associated with decreased levels of proinflammatory cytokines and reduced lung pathology. Compensation by other neuropeptides/hormones or changes in VIP receptors could certainly occur in global VIP KO. These mice also have increased levels of systemic IL-10 which might affect responsiveness to LPS. Interestingly, myeloid peritoneal cells from VIP KOs were impaired in their response to LPS, exhibiting reduced NFkB activation [111]. Presently, the reason for this impairment is not understood. A blunted response to LPS could also result in the so-called endotoxin tolerance [112] which would maintain and amplify the lack of response to LPS. Whether the VIP KO mice also exhibit resistance to CLP-induced sepsis has not been reported yet. In any case, definite answers related to the role of endogenous VIP in sepsis will have to wait for the development of conditional temporal and cell-specific VIP KOs.

Inflammatory Bowel Disease (IBD)

The term IBD includes Crohn's disease (CD) and ulcerative colitis (UC), two chronic and relapsing diseases which share common traits but also have distinct clinical and pathological features. IBD is characterized by increased inflammation, disrupted epithelial and mucosal barrier, loss of tolerance to commensal bacteria, and a generally dysregulated immune response. The inflammatory profile is different in CD versus UC, with Th1/Th17 cytokines such as IFNγ, IL-2 and IL-17 as primary mediators in CD, and Th2 cytokines such as IL-4, IL-5, and IL-10 as major

mediators in the chronic phase of UC. A number of IBD animal models, including chemically induced, genetically engineered mice including knockouts and transgenics, as well as adoptive transfer models, have been developed [113]. Although none of these models are the exact counterparts of human CD or UC, the two major chemically induced colitis models used to test the effects of VIP, the dextran sodium sulfate (DSS) and the trinitrobenzene sulfonic acid (TNBS) models, are considered to resemble human UC and CD, respectively [114].

VIP Effects in DSS Colitis

In general, the role of VIP in IBD as tested in animal models has been controversial. Although early experiments using the TNBS model showed a protective effect [115–118], later reports using continuous infusion of VIP showed no effect, and VIP-deficient mice were reported to exhibit reduced pathology [119, 120]. Similar results were obtained in the DSS colitis model, with VIP KOs being remarkably resistant, and wild-type mice injected with a pan-VIP receptor or a VPAC1 antagonist exhibiting reduced clinical colitis [121].

Differences in VPAC1/VPAC2 expression and function could provide an explanation for these confounding results. According to existing information, VPAC1 is constitutively expressed, whereas VPAC2 is induced following activation. A detailed analysis of VPAC1/VPAC2 levels should provide information on the dominant receptor at different stages of activation in immune cell subsets.

In terms of function, recent studies using VIP receptor knockouts (Table 5.1) identified interesting differences. A comparison between VPAC2 KO and T cell-specific transgenic hVPAC2 mice established that VPAC2 was responsible for the VIP-induced shift in favor of Th2 cells [81, 82]. More recently, Tan et al. [122] reported increased EAE severity in VPAC2-deficient mice, associated with higher levels of IFNγ and IL-17, reduced levels of IL-10 and IL-4, and reduced numbers of Foxp3+ Treg in lymph nodes, thymus, and CNS. This strongly suggests that VPAC2 is the mediator of VIP effects in T cell differentiation, exerting an anti-inflammatory effect through an increase in Th2/Treg subsets at the expense of the proinflammatory Th1/Th17 cells. The role of VPAC1 on the other hand is less clear. In vitro experiments using macrophage and microglia cultures pointed to VPAC1 as the receptor mediating the anti-inflammatory effects of VIP [123–125]. However, its role in vivo has not been defined. Recently, a comparison between VPAC1- and VPAC2-KOs identified an opposite pattern in acute DSS colitis, with much milder clinical symptoms in VPAC1-KO and significantly increased severity in VPAC2-KO, suggesting that VPAC1 signaling exacerbates, whereas VPAC2 signaling attenuates clinical disease [126]. Based on differences in mucosal cytokines, neutrophils, and MMPs, the authors concluded that VPAC1 mediates an increased recruitment of immune cells to lymph nodes and Peyer's patches and proposed that the VIP → VPAC1 axis acts as a major mediator for pathogenesis, emphasizing the importance of the VPAC1/VPAC2 ratio in determining whether VIP exerts a protective or a pathogenic effect.

VIP Effects in TNBS Colitis

TNBS colitis develops as a delayed-type hypersensitivity reaction to hapten-protein complexes and is characterized by inflammatory infiltrates consisting of CD4 and CD8 T cells, macrophages, and granulocytes, the presence of irregular crypts, and loss of goblet cells [127]. The effects of VIP in TNBS colitis are controversial. Gomariz and colleagues were the first to describe the effects of VIP administration in TNBS colitis and reported significant reductions in weight loss and intestinal inflammation, associated with downregulation of IL-1β and TNFα in colon extracts and serum, and reduced IFNγ expression in splenic and lamina propria CD4 T cells [116]. The same group reported colonic downregulation of proinflammatory cytokines/chemokines and of TLR2 and 4, as well as upregulation of IL-10 and IL-4 in TNBS mice treated with VIP [58, 115]. In contrast to these results, continuous VIP delivery through osmotic pumps in a severe TNBS model did not affect weight loss and mortality or the Th1/Th2 profile [120]. The conflicting results might reflect differences in terms of mild versus severe inflammation or continuous versus intermittent exposure to VIP.

Surprisingly, compared to wild-type controls, VIP-deficient mice developed a milder, instead of an exacerbated, clinical profile (Table 5.1) [119]. This is reminiscent of results obtained for VIP KO in EAE, endotoxemic shock, and DSS colitis [111, 121, 128]. Interestingly, as expected, in all these instances, VIP KO T cell proliferation is significantly increased, confirming that lack of VIP leads to hyperresponsive T cells. The question remains whether APCs develop normally in the absence of VIP. It has been reported that peritoneal myeloid cells from VIP KO have a blunted response to LPS [111]. This could be due to alterations in TLRs, changes in intracellular signaling molecules, and alterations in the production of cytokines and chemokines. Also, if the VIP → VPAC1 axis is proinflammatory as proposed by Yadav et al. [126], lack of endogenous VIP could indeed be beneficial by impairing leukocyte traffic. In TNBS wild-type mice, the beneficial effect of exogenous VIP administration could be the result of preferential initiation of the anti-inflammatory VIP → VPAC2 axis in the presence of large amounts of VIP and concomitant upregulation of VPAC2 on activated immune cells.

VIP: Therapeutic Perspectives

Based on its immunosuppressive effect on multiple cellular and molecular targets, VIP is a potential therapeutic agent for inflammatory/autoimmune diseases. However, due to issues related primarily to stability and delivery, VIP is presently used only in treatment of pulmonary hypertension and sarcoidosis where it is delivered as an inhalant [93, 129]. Several options for increasing stability, such as amino acid substitutions, use of liposomes or of silver-protected nanoparticles for delivery, combined treatments with peptidase inhibitors, or serum neuropeptide-binding proteins, are currently under investigation (reviewed in [130]). In terms of localized

VIP expression and release, VIP gene therapy is another option, especially since its success in CIA therapy [97]. Unfortunately, however, this method lacks tissue and cell specificity. Cellular therapy using VIP-transduced DC has the advantage of developing tolerogenic DC in vitro with the capacity to induce antigen-specific Treg in vivo. Although this approach still faces several challenges especially in the identification of disease-specific autoantigens, the potential of generating long-lived antigen-specific Treg in vivo is a great advantage.

Although VIP is a major endogenous neuropeptide in the GI tract, its potential use in IBD therapy is presently limited by conflicting data obtained in wild-type versus VIP KOs. VIP treatment in TNBS colitis led to attenuated disease [58, 115, 116], whereas the VIP KOs expected to be highly susceptible were instead resistant to TNBS colitis [119]. This was also true for EAE and for LPS endotoxemia [111, 128]. The fact that VPAC1 and VPAC2, the two receptors which bind VIP with equal affinity, appear to have opposite effects in DSS colitis [126] might explain the contradiction. Future studies are required to investigate the differential contributions of the two receptors and changes in the VPAC1/VPAC2 ratio in different cell types at different disease stages. Meantime, PACAP, the neuropeptide structurally and functionally similar to VIP, could represent a better therapeutic target, since PACAP treatment proved beneficial in sepsis and IBD models [117, 131], and as expected, PACAP KOs developed exacerbated DSS colitis [132]. The effect of PACAP deficiency in other models of autoimmune/inflammatory diseases remains to be determined.

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Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Chandrasekharan B, Nezami BG, Srinivasan S. Emerging neuropeptide targets in inflammation: NPY and VIP. Am J Physiol Gastrointest Liver Physiol. 2013;304:G949–57.
2. Margolis KG, Gershon MD. Neuropeptides and inflammatory bowel disease. Curr Opin Gastroenterol. 2009;25:503–11.
3. Harmar AJ, Fahrenkrug J, Gozes I, Laburthe M, May V, Pisegna JR, Vaudry D, Vaudry H, Waschek JA, Said SI. Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: Iuphar review 1. Br J Pharmacol. 2012;166:4–17.
4. Furness JB. Types of neurons in the enteric nervous system. J Auton Nerv Syst. 2000; 81:87–96.
5. Banks MR, Farthing MJ, Robberecht P, Burleigh DE. Antisecretory actions of a novel vasoactive intestinal polypeptide (vip) antagonist in human and rat small intestine. Br J Pharmacol. 2005;144:994–1001.
6. Cooke HJ. Neurotransmitters in neuronal reflexes regulating intestinal secretion. Ann N Y Acad Sci. 2000;915:77–80.
7. Fung C, Unterweger P, Parry LJ, Bornstein JC, Foong JP. Vpac1 receptors regulate intestinal secretion and muscle contractility by activating cholinergic neurons in guinea pig jejunum. Am J Physiol Gastrointest Liver Physiol. 2014;306:G748–58.

8. Katsoulis S, Schmidt WE, Clemens A, Schworer H, Creutzfeldt W. Vasoactive intestinal polypeptide induces neurogenic contraction of guinea-pig ileum. Involvement of acetylcholine and substance p. Regul Pept. 1992;38:155–64.
9. Reddix R, Kuhawara A, Wallace L, Cooke HJ. Vasoactive intestinal polypeptide: a transmitter in submucous neurons mediating secretion in guinea pig distal colon. J Pharmacol Exp Ther. 1994;269:1124–9.
10. Shi XZ, Sarna SK. Gene therapy of cav1.2 channel with vip and vip receptor agonists and antagonists: a novel approach to designing promotility and antimotility agents. Am J Physiol Gastrointest Liver Physiol. 2008;295:G187–96.
11. Lelievre V, Favrais G, Abad C, Adle-Biassette H, Lu Y, Germano PM, Cheung-Lau G, Pisegna JR, Gressens P, Lawson G, Waschek JA. Gastrointestinal dysfunction in mice with a targeted mutation in the gene encoding vasoactive intestinal polypeptide: a model for the study of intestinal ileus and hirschsprung's disease. Peptides. 2007;28:1688–99.
12. Holland PW, Garcia-Fernandez J, Williams NA, Sidow A. Gene duplications and the origins of vertebrate development. Dev Suppl. 1994;125–133.
13. Hoyle CH. Neuropeptide families: evolutionary perspectives. Regul Pept. 1998;73:1–33.
14. Du BH, Eng J, Hulmes JD, Chang M, Pan YC, Yalow RS. Guinea pig has a unique mammalian vip. Biochem Biophys Res Commun. 1985;128:1093–8.
15. Foster N. Editorial: vasoactive intestinal peptide (vip): historic perspective and future potential. Endocr Metab Immune Disord Drug Targets. 2012;12:303–7.
16. Henning RJ, Sawmiller DR. Vasoactive intestinal peptide: cardiovascular effects. Cardiovasc Res. 2001;49:27–37.
17. Bellinger DL, Lorton D, Horn L, Brouxhon S, Felten SY, Felten DL. Vasoactive intestinal polypeptide (vip) innervation of rat spleen, thymus, and lymph nodes. Peptides. 1997;18: 1139–49.
18. Ganea D, Hooper KM, Kong W. The neuropeptide vasoactive intestinal peptide: direct effects on immune cells and involvement in inflammatory and autoimmune diseases. Acta Physiol (Oxf). 2014.
19. Leceta J, Martinez MC, Delgado M, Garrido E, Gomariz RP. Lymphoid cell subpopulations containing vasoactive intestinal peptide in the rat. Peptides. 1994;15:791–7.
20. Delgado M, Ganea D. Cutting edge: is vasoactive intestinal peptide a type 2 cytokine? J Immunol. 2001;166:2907–12.
21. Voice JK, Grinninger C, Kong Y, Bangale Y, Paul S, Goetzl EJ. Roles of vasoactive intestinal peptide (vip) in the expression of different immune phenotypes by wild-type mice and t cell-targeted type ii vip receptor transgenic mice. J Immunol. 2003;170:308–14.
22. Li JM, Southerland L, Hossain MS, Giver CR, Wang Y, Darlak K, Harris W, Waschek J, Waller EK. Absence of vasoactive intestinal peptide expression in hematopoietic cells enhances th1 polarization and antiviral immunity in mice. J Immunol. 2011;187:1057–65.
23. Andersen O, Fahrenkrug J, Wikkelso C, Johansson BB. Vip in cerebrospinal fluid of patients with multiple sclerosis. Peptides. 1984;5:435–7.
24. Martinez C, Ortiz AM, Juarranz Y, Lamana A, Seoane IV, Leceta J, Garcia-Vicuna R, Gomariz RP, Gonzalez-Alvaro I. Serum levels of vasoactive intestinal peptide as a prognostic marker in early arthritis. PLoS One. 2014;9:e85248.
25. Tornwall J, Uusitalo H, Hukkanen M, Sorsa T, Konttinen YT. Distribution of vasoactive intestinal peptide (vip) and its binding sites in labial salivary glands in sjogren's syndrome and in normal controls. Clin Exp Rheumatol. 1994;12:287–92.
26. Bangale Y, Karle S, Planque S, Zhou YX, Taguchi H, Nishiyama Y, Li L, Kalaga R, Paul S. Vipase autoantibodies in fas-defective mice and patients with autoimmune disease. FASEB J. 2003;17:628–35.
27. Bishop AE, Polak JM, Bryant MG, Bloom SR, Hamilton S. Abnormalities of vasoactive intestinal polypeptide-containing nerves in crohn's disease. Gastroenterology. 1980;79:853–60.
28. O'Morain C, Bishop AE, McGregor GP, Levi AJ, Bloom SR, Polak JM, Peters TJ. Vasoactive intestinal peptide concentrations and immunocytochemical studies in rectal biopsies from patients with inflammatory bowel disease. Gut. 1984;25:57–61.

29. Schneider J, Jehle EC, Starlinger MJ, Neunlist M, Michel K, Hoppe S, Schemann M. Neurotransmitter coding of enteric neurones in the submucous plexus is changed in non-inflamed rectum of patients with crohn's disease. Neurogastroenterol Motil. 2001;13:255–64.
30. Kimura M, Masuda T, Hiwatashi N, Toyota T, Nagura H. Changes in neuropeptide-containing nerves in human colonic mucosa with inflammatory bowel disease. Pathol Int. 1994;44: 624–34.
31. Kubota Y, Petras RE, Ottaway CA, Tubbs RR, Farmer RG, Fiocchi C. Colonic vasoactive intestinal peptide nerves in inflammatory bowel disease. Gastroenterology. 1992;102: 1242–51.
32. Baticic L, Detel D, Kucic N, Buljevic S, Pugel EP, Varljen J. Neuroimmunomodulative properties of dipeptidyl peptidase iv/cd26 in a tnbs-induced model of colitis in mice. J Cell Biochem. 2011;112:3322–33.
33. Sigalet DL, Wallace L, De Heuval E, Sharkey KA. The effects of glucagon-like peptide 2 on enteric neurons in intestinal inflammation. Neurogastroenterol Motil. 2010;22:1318–e1350.
34. Abad C, Gomariz R, Waschek J, Leceta J, Martinez C, Juarranz Y, Arranz A. Vip in inflammatory bowel disease: state of the art. Endocr Metab Immune Disord Drug Targets. 2012;12:316–22.
35. Reubi JC. In vitro evaluation of vip/pacap receptors in healthy and diseased human tissues. Clinical implications. Ann N Y Acad Sci. 2000;921:1–25.
36. Usdin TB, Bonner TI, Mezey E. Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. Endocrinology. 1994;135:2662–80.
37. Vaudry D, Gonzalez BJ, Basille M, Yon L, Fournier A, Vaudry H. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. Pharmacol Rev. 2000;52:269–324.
38. Couvineau A, Laburthe M. Vpac receptors: structure, molecular pharmacology and interaction with accessory proteins. Br J Pharmacol. 2012;166:42–50.
39. Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. Pharmacol Rev. 2004;56:249–90.
40. Delgado M, Ganea D. Anti-inflammatory neuropeptides: a new class of endogenous immunoregulatory agents. Brain Behav Immun. 2008;22:1146–51.
41. Gonzalez-Rey E, Delgado M. Anti-inflammatory neuropeptide receptors: new therapeutic targets for immune disorders? Trends Pharmacol Sci. 2007;28:482–91.
42. Delgado M, Robledo G, Rueda B, Varela N, O'Valle F, Hernandez-Cortes P, Caro M, Orozco G, Gonzalez-Rey E, Martin J. Genetic association of vasoactive intestinal peptide receptor with rheumatoid arthritis: altered expression and signal in immune cells. Arthritis Rheum. 2008;58:1010–9.
43. Juarranz Y, Gutierrez-Canas I, Santiago B, Carrion M, Pablos JL, Gomariz RP. Differential expression of vasoactive intestinal peptide and its functional receptors in human osteoarthritic and rheumatoid synovial fibroblasts. Arthritis Rheum. 2008;58:1086–95.
44. Paladini F, Cocco E, Cauli A, Cascino I, Vacca A, Belfiore F, Fiorillo MT, Mathieu A, Sorrentino R. A functional polymorphism of the vasoactive intestinal peptide receptor 1 gene correlates with the presence of hla-b*2705 in sardinia. Genes Immun. 2008;9:659–67.
45. Sun W, Hong J, Zang YC, Liu X, Zhang JZ. Altered expression of vasoactive intestinal peptide receptors in t lymphocytes and aberrant th1 immunity in multiple sclerosis. Int Immunol. 2006;18:1691–700.
46. Hauk V, Fraccaroli L, Grasso E, Eimon A, Ramhorst R, Hubscher O, Perez Leiros C. Monocytes from sjogren's syndrome patients display increased vasoactive intestinal peptide receptor 2 expression and impaired apoptotic cell phagocytosis. Clin Exp Immunol. 2014;177:662–70.
47. Harmar AJ, Arimura A, Gozes I, Journot L, Laburthe M, Pisegna JR, Rawlings SR, Robberecht P, Said SI, Sreedharan SP, Wank SA, Waschek JA. International union of pharmacology. Xviii. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Pharmacol Rev. 1998;50:265–70.
48. Massacand JC, Kaiser P, Ernst B, Tardivel A, Burki K, Schneider P, Harris NL. Intestinal bacteria condition dendritic cells to promote iga production. PLoS One. 2008;3:e2588.

49. Delgado M, Chorny A, Gonzalez-Rey E, Ganea D. Vasoactive intestinal peptide generates cd4+cd25+ regulatory t cells in vivo. J Leukoc Biol. 2005;78:1327–38.
50. Delgado M, Gonzalez-Rey E, Ganea D. The neuropeptide vasoactive intestinal peptide generates tolerogenic dendritic cells. J Immunol. 2005;175:7311–24.
51. Yukawa T, Oshitani N, Yamagami H, Watanabe K, Higuchi K, Arakawa T. Differential expression of vasoactive intestinal peptide receptor 1 expression in inflammatory bowel disease. Int J Mol Med. 2007;20:161–7.
52. Burian B, Storka A, Marzluf BA, Yen YC, Lambers C, Robibaro B, Vonbank K, Mosgoeller W, Petkov V. Vasoactive intestinal peptide (vip) receptor expression in monocyte-derived macrophages from copd patients. Peptides. 2010;31:603–8.
53. Kawashima K, Yoshikawa K, Fujii YX, Moriwaki Y, Misawa H. Expression and function of genes encoding cholinergic components in murine immune cells. Life Sci. 2007;80:2314–9.
54. Leceta J, Gomariz RP, Martinez C, Abad C, Ganea D, Delgado M. Receptors and transcriptional factors involved in the anti-inflammatory activity of vip and pacap. Ann N Y Acad Sci. 2000;921:92–102.
55. Chorny A, Delgado M. Neuropeptides rescue mice from lethal sepsis by down-regulating secretion of the late-acting inflammatory mediator high mobility group box 1. Am J Pathol. 2008;172:1297–307.
56. Arranz A, Juarranz Y, Leceta J, Gomariz RP, Martinez C. Vip balances innate and adaptive immune responses induced by specific stimulation of tlr2 and tlr4. Peptides. 2008;29:948–56.
57. Foster N, Lea SR, Preshaw PM, Taylor JJ. Pivotal advance: vasoactive intestinal peptide inhibits up-regulation of human monocyte tlr2 and tlr4 by lps and differentiation of monocytes to macrophages. J Leukoc Biol. 2007;81:893–903.
58. Gomariz RP, Arranz A, Abad C, Torroba M, Martinez C, Rosignoli F, Garcia-Gomez M, Leceta J, Juarranz Y. Time-course expression of toll-like receptors 2 and 4 in inflammatory bowel disease and homeostatic effect of vip. J Leukoc Biol. 2005;78:491–502.
59. Gutierrez-Canas I, Juarranz Y, Santiago B, Arranz A, Martinez C, Galindo M, Paya M, Gomariz RP, Pablos JL. Vip down-regulates tlr4 expression and tlr4-mediated chemokine production in human rheumatoid synovial fibroblasts. Rheumatology (Oxford). 2006;45:527–32.
60. Delgado M, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit nuclear factor-kappa b-dependent gene activation at multiple levels in the human monocytic cell line thp-1. J Biol Chem. 2001;276:369–80.
61. Delgado M, Ganea D. Inhibition of ifn-gamma-induced janus kinase-1-stat1 activation in macrophages by vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. J Immunol. 2000;165:3051–7.
62. Delgado M, Munoz-Elias EJ, Kan Y, Gozes I, Fridkin M, Brenneman DE, Gomariz RP, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit tumor necrosis factor alpha transcriptional activation by regulating nuclear factor-kb and camp response element-binding protein/c-jun. J Biol Chem. 1998;273:31427–36.
63. Delgado M. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the mekk1/mek4/jnk signaling pathway in endotoxin-activated microglia. Biochem Biophys Res Commun. 2002;293:771–6.
64. Maldonado RA, von Andrian UH. How tolerogenic dendritic cells induce regulatory t cells. Adv Immunol. 2010;108:111–65.
65. Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Pozo D, Ganea D, Delgado M. Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. Proc Natl Acad Sci U S A. 2005;102:13562–7.
66. Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Ganea D, Delgado M. Vasoactive intestinal peptide induces regulatory dendritic cells that prevent acute graft-versus-host disease while maintaining the graft-versus-tumor response. Blood. 2006;107:3787–94.
67. Toscano MG, Delgado M, Kong W, Martin F, Skarica M, Ganea D. Dendritic cells transduced with lentiviral vectors expressing vip differentiate into vip-secreting tolerogenic-like dcs. Mol Ther. 2010;18:1035–45.

68. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 2003;3:331–41.
69. Ruane DT, Lavelle EC. The role of cd103(+) dendritic cells in the intestinal mucosal immune system. Front Immunol. 2011;2:25.
70. Scott CL, Aumeunier AM, Mowat AM. Intestinal cd103+ dendritic cells: master regulators of tolerance? Trends Immunol. 2011;32:412–9.
71. Scott CL, Bain CC, Wright PB, Sichien D, Kotarsky K, Persson EK, Luda K, Guilliams M, Lambrecht BN, Agace WW, Milling SW, Mowat AM. Ccr2cd103 intestinal dendritic cells develop from dc-committed precursors and induce interleukin-17 production by t cells. Mucosal Immunol. 2015;8:327–39.
72. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from cx3cr1(+) macrophages to cd103(+) dendritic cells. Immunity. 2014;40:248–61.
73. Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW, Pabst O. Intestinal cd103+, but not cx3cr1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. J Exp Med. 2009;206:3101–14.
74. Delgado M, Reduta A, Sharma V, Ganea D. Vip/pacap oppositely affects immature and mature dendritic cell expression of cd80/cd86 and the stimulatory activity for cd4(+) t cells. J Leukoc Biol. 2004;75:1122–30.
75. Delgado M, Sun W, Leceta J, Ganea D. Vip and pacap differentially regulate the costimulatory activity of resting and activated macrophages through the modulation of b7.1 and b7.2 expression. J Immunol. 1999;163:4213–23.
76. Ganea D, Rodriguez R, Delgado M. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: players in innate and adaptive immunity. Cell Mol Biol (Noisy-le-Grand). 2003;49:127–42.
77. Liu L, Yen JH, Ganea D. A novel vip signaling pathway in t cells camp--> protein tyrosine phosphatase (shp-2?)--> jak2/stat4--> th1 differentiation. Peptides. 2007;28:1814–24.
78. Voice J, Donnelly S, Dorsam G, Dolganov G, Paul S, Goetzl EJ. C-maf and junb mediation of th2 differentiation induced by the type 2 g protein-coupled receptor (vpac2) for vasoactive intestinal peptide. J Immunol. 2004;172:7289–96.
79. Delgado M, Leceta J, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide promote in vivo generation of memory th2 cells. FASEB J. 2002;16:1844–6.
80. Sharma V, Delgado M, Ganea D. Granzyme b, a new player in activation-induced cell death, is down-regulated by vasoactive intestinal peptide in th2 but not th1 effectors. J Immunol. 2006;176:97–110.
81. Goetzl EJ, Voice JK, Shen S, Dorsam G, Kong Y, West KM, Morrison CF, Harmar AJ. Enhanced delayed-type hypersensitivity and diminished immediate-type hypersensitivity in mice lacking the inducible vpac(2) receptor for vasoactive intestinal peptide. Proc Natl Acad Sci U S A. 2001;98:13854–9.
82. Voice JK, Dorsam G, Lee H, Kong Y, Goetzl EJ. Allergic diathesis in transgenic mice with constitutive t cell expression of inducible vasoactive intestinal peptide receptor. FASEB J. 2001;15:2489–96.
83. Bovenschen HJ, van de Kerkhof PC, van Erp PE, Woestenenk R, Joosten I, Koenen HJ. Foxp3+ regulatory t cells of psoriasis patients easily differentiate into il-17a-producing cells and are found in lesional skin. J Invest Dermatol. 2011;131:1853–60.
84. Ferraccioli G, Zizzo G. The potential role of th17 in mediating the transition from acute to chronic autoimmune inflammation: rheumatoid arthritis as a model. Discov Med. 2011;11:413–24.
85. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. Gut. 2003;52:65–70.
86. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human th17 lymphocytes promote blood–brain barrier disruption and central nervous system inflammation. Nat Med. 2007;13:1173–5.

87. Jimeno R, Gomariz RP, Gutierrez-Canas I, Martinez C, Juarranz Y, Leceta J. New insights into the role of vip on the ratio of t-cell subsets during the development of autoimmune diabetes. Immunol Cell Biol. 2010;88:734–45.
88. Deng S, Xi Y, Wang H, Hao J, Niu X, Li W, Tao Y, Chen G. Regulatory effect of vasoactive intestinal peptide on the balance of treg and th17 in collagen-induced arthritis. Cell Immunol. 2010;265:105–10.
89. Ding W, Manni M, Stohl LL, Zhou XK, Wagner JA, Granstein RD. Pituitary adenylate cyclase-activating peptide and vasoactive intestinal polypeptide bias langerhans cell ag presentation toward th17 cells. Eur J Immunol. 2012;42:901–11.
90. Yadav M, Rosenbaum J, Goetzl EJ. Cutting edge: vasoactive intestinal peptide (vip) induces differentiation of th17 cells with a distinctive cytokine profile. J Immunol. 2008;180: 2772–6.
91. Jimeno R, Leceta J, Martinez C, Gutierrez-Canas I, Carrion M, Perez-Garcia S, Garin M, Mellado M, Gomariz RP, Juarranz Y. Vasoactive intestinal peptide maintains the nonpathogenic profile of human th17-polarized cells. J Mol Neurosci. 2014;54:512–25.
92. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, Wu C, Kleinewietfeld M, Kunder S, Hafler DA, Sobel RA, Regev A, Kuchroo VK. Induction and molecular signature of pathogenic th17 cells. Nat Immunol. 2012;13:991–9.
93. Prasse A, Zissel G, Lutzen N, Schupp J, Schmiedlin R, Gonzalez-Rey E, Rensing-Ehl A, Bacher G, Cavalli V, Bevec D, Delgado M, Muller-Quernheim J. Inhaled vasoactive intestinal peptide exerts immunoregulatory effects in sarcoidosis. Am J Respir Crit Care Med. 2010;182:540–8.
94. Chen G, Hao J, Xi Y, Wang W, Wang Z, Li N, Li W. The therapeutic effect of vasoactive intestinal peptide on experimental arthritis is associated with cd4+cd25+ t regulatory cells. Scand J Immunol. 2008;68:572–8.
95. Fernandez-Martin A, Gonzalez-Rey E, Chorny A, Martin J, Pozo D, Ganea D, Delgado M. Vip prevents experimental multiple sclerosis by downregulating both inflammatory and autoimmune components of the disease. Ann N Y Acad Sci. 2006;1070:276–81.
96. Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Delgado M. Vasoactive intestinal peptide induces cd4+, cd25+ t regulatory cells with therapeutic effect in collagen-induced arthritis. Arthritis Rheum. 2006;54:864–76.
97. Delgado M, Toscano MG, Benabdellah K, Cobo M, O'Valle F, Gonzalez-Rey E, Martin F. In vivo delivery of lentiviral vectors expressing vasoactive intestinal peptide complementary DNA as gene therapy for collagen-induced arthritis. Arthritis Rheum. 2008;58:1026–37.
98. DMaG D. Vasoactive intestinal peptide: immune mediator and potential therapeutic agent. In: Levite M, editor. Nerve-driven immunity. Wien: Springer; 2012. p. 257–89.
99. Cauwels A, Brouckaert P. Nitrite regulation of shock. Cardiovasc Res. 2011;89:553–9.
100. Remick DG, Bolgos G, Copeland S, Siddiqui J. Role of interleukin-6 in mortality from and physiologic response to sepsis. Infect Immun. 2005;73:2751–7.
101. Tang H, Ivanciu L, Popescu N, Peer G, Hack E, Lupu C, Taylor Jr FB, Lupu F. Sepsis-induced coagulation in the baboon lung is associated with decreased tissue factor pathway inhibitor. Am J Pathol. 2007;171:1066–77.
102. Ulloa L, Tracey KJ. The "cytokine profile": a code for sepsis. Trends Mol Med. 2005;11:56–63.
103. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. Hmg-1 as a late mediator of endotoxin lethality in mice. Science. 1999;285:248–51.
104. Fink MP. Animal models of sepsis. Virulence. 2014;5:143–53.
105. Unsinger J, McDonough JS, Shultz LD, Ferguson TA, Hotchkiss RS. Sepsis-induced human lymphocyte apoptosis and cytokine production in "humanized" mice. J Leukoc Biol. 2009; 86:219–27.
106. Ibrahim H, Barrow P, Foster N. Vip as a potential therapeutic agent in gram negative sepsis. Endocr Metab Immune Disord Drug Targets. 2012;12:308–15.

107. Delgado M, Gomariz RP, Martinez C, Abad C, Leceta J. Anti-inflammatory properties of the type 1 and type 2 vasoactive intestinal peptide receptors: role in lethal endotoxic shock. Eur J Immunol. 2000;30:3236–46.
108. Delgado M, Martinez C, Pozo D, Calvo JR, Leceta J, Ganea D, Gomariz RP. Vasoactive intestinal peptide (vip) and pituitary adenylate cyclase-activation polypeptide (pacap) protect mice from lethal endotoxemia through the inhibition of tnf-alpha and il-6. J Immunol. 1999;162:1200–5.
109. Martinez C, Abad C, Delgado M, Arranz A, Juarranz MG, Rodriguez-Henche N, Brabet P, Leceta J, Gomariz RP. Anti-inflammatory role in septic shock of pituitary adenylate cyclase-activating polypeptide receptor. Proc Natl Acad Sci U S A. 2002;99:1053–8.
110. Martinez C, Juarranz Y, Abad C, Arranz A, Miguel BG, Rosignoli F, Leceta J, Gomariz RP. Analysis of the role of the pac1 receptor in neutrophil recruitment, acute-phase response, and nitric oxide production in septic shock. J Leukoc Biol. 2005;77:729–38.
111. Abad C, Tan YV, Cheung-Lau G, Nobuta H, Waschek JA. Vip deficient mice exhibit resistance to lipopolysaccharide induced endotoxemia with an intrinsic defect in proinflammatory cellular responses. PLoS One. 2012;7:e36922.
112. Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol. 2009;30:475–87.
113. Goyal N, Rana A, Ahlawat A, Bijjem KR, Kumar P. Animal models of inflammatory bowel disease: a review. Inflammopharmacology. 2014;22:219–33.
114. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol. 2014;18:279–88.
115. Abad C, Juarranz Y, Martinez C, Arranz A, Rosignoli F, Garcia-Gomez M, Leceta J, Gomariz RP. Cdna array analysis of cytokines, chemokines, and receptors involved in the development of tnbs-induced colitis: homeostatic role of vip. Inflamm Bowel Dis. 2005;11:674–84.
116. Abad C, Martinez C, Juarranz MG, Arranz A, Leceta J, Delgado M, Gomariz RP. Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of crohn's disease. Gastroenterology. 2003;124:961–71.
117. Arranz A, Abad C, Juarranz Y, Leceta J, Martinez C, Gomariz RP. Vasoactive intestinal peptide as a healing mediator in crohn's disease. Neuroimmunomodulation. 2008;15:46–53.
118. Arranz A, Abad C, Juarranz Y, Torroba M, Rosignoli F, Leceta J, Gomariz RP, Martinez C. Effect of vip on tlr2 and tlr4 expression in lymph node immune cells during tnbs-induced colitis. Ann N Y Acad Sci. 2006;1070:129–34.
119. Abad C, Cheung-Lau G, Coute-Monvoisin AC, Waschek JA. Vasoactive intestinal peptide-deficient mice exhibit reduced pathology in trinitrobenzene sulfonic acid-induced colitis. Neuroimmunomodulation. 2015;22:203–12.
120. Newman R, Cuan N, Hampartzoumian T, Connor SJ, Lloyd AR, Grimm MC. Vasoactive intestinal peptide impairs leucocyte migration but fails to modify experimental murine colitis. Clin Exp Immunol. 2005;139:411–20.
121. Vu JP, Million M, Larauche M, Luong L, Norris J, Waschek JA, Pothoulakis C, Pisegna JR, Germano PM. Inhibition of vasoactive intestinal polypeptide (vip) induces resistance to dextran sodium sulfate (dss)-induced colitis in mice. J Mol Neurosci. 2014;52:37–47.
122. Tan YV, Abad C, Wang Y, Lopez R, Waschek JA. Vpac2 (vasoactive intestinal peptide receptor type 2) receptor deficient mice develop exacerbated experimental autoimmune encephalomyelitis with increased th1/th17 and reduced th2/treg responses. Brain Behav Immun. 2015;44:167–75.
123. Delgado M, Ganea D. Inhibition of endotoxin-induced macrophage chemokine production by vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide in vitro and in vivo. J Immunol. 2001;167:966–75.
124. Delgado M, Leceta J, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the production of inflammatory mediators by activated microglia. J Leukoc Biol. 2003;73:155–64.

125. Delgado M, Munoz-Elias EJ, Gomariz RP, Ganea D. Vip and pacap inhibit il-12 production in lps-stimulated macrophages. Subsequent effect on ifngamma synthesis by t cells. J Neuroimmunol. 1999;96:167–81.
126. Yadav M, Huang MC, Goetzl EJ. Vpac1 (vasoactive intestinal peptide (vip) receptor type 1) g protein-coupled receptor mediation of vip enhancement of murine experimental colitis. Cell Immunol. 2011;267:124–32.
127. Kremer B, Mariman R, van Erk M, Lagerweij T, Nagelkerken L. Temporal colonic gene expression profiling in the recurrent colitis model identifies early and chronic inflammatory processes. PLoS One. 2012;7:e50388.
128. Abad C, Tan YV, Lopez R, Nobuta H, Dong H, Phan P, Feng JM, Campagnoni AT, Waschek JA. Vasoactive intestinal peptide loss leads to impaired cns parenchymal t-cell infiltration and resistance to experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2010;107:19555–60.
129. Leuchte HH, Baezner C, Baumgartner RA, Bevec D, Bacher G, Neurohr C, Behr J. Inhalation of vasoactive intestinal peptide in pulmonary hypertension. Eur Respir J. 2008;32:1289–94.
130. Gonzalez-Rey E, Ganea D, Delgado M. Neuropeptides: keeping the balance between pathogen immunity and immune tolerance. Curr Opin Pharmacol. 2010;10:473–81.
131. Azuma YT, Hagi K, Shintani N, Kuwamura M, Nakajima H, Hashimoto H, Baba A, Takeuchi T. Pacap provides colonic protection against dextran sodium sulfate induced colitis. J Cell Physiol. 2008;216:111–9.
132. Nemetz N, Abad C, Lawson G, Nobuta H, Chhith S, Duong L, Tse G, Braun J, Waschek JA. Induction of colitis and rapid development of colorectal tumors in mice deficient in the neuropeptide pacap. Int J Cancer. 2008;122:1803–9.
133. Tan YV, Abad C, Lopez R, Dong H, Liu S, Lee A, Gomariz RP, Leceta J, Waschek JA. Pituitary adenylyl cyclase-activating polypeptide is an intrinsic regulator of treg abundance and protects against experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2009;106:2012–7.
134. Tan YV, Abad C, Wang Y, Lopez R, Waschek JA. Pituitary adenylate cyclase activating peptide deficient mice exhibit impaired thymic and extrathymic regulatory t cell proliferation during eae. PLoS One. 2013;8:e61200.

Chapter 6
Helicobacter pylori, Experimental Autoimmune Encephalomyelitis, and Multiple Sclerosis

Karen Robinson, Joanna Stephens, Cris S. Constantinescu, and Bruno Gran

Abstract *Helicobacter pylori* (*H. pylori*) is a common human pathogen which has been implicated in the pathogenesis of peptic ulcer disease and stomach cancer. *H. pylori* colonizes the stomach of about half the globe's population, and its decline in the developed world coincided temporally with an increase in autoimmune and inflammatory disease. The hygiene hypothesis or "old friends" hypothesis have been proposed to explain this inverse link. Indeed, while *H. pylori* affects the innate immune system and induces strong cellular and humoral immune responses, it also has developed the ability to induce strong regulatory immune mechanisms to allow its persistence; these include, but are not restricted to, regulatory T cells (Treg cells).

Epidemiological and experimental evidence suggests a protective effect on autoimmune and inflammatory conditions including asthma, inflammatory bowel disease, and multiple sclerosis. The mechanisms of this protective effect are likely to be complex and include Treg cells, other immunoregulatory processes, and other host- and *H. pylori*-associated factors. Some of these have been explored in studies

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in the experimental autoimmune encephalomyelitis models and are currently being investigated in multiple sclerosis.

On the other hand, a positive association has been found between *H. pylori* and neuromyelitis optica.

This chapter reviews and discusses the immune response to *H. pylori*, with emphasis with immunoregulatory mechanisms, its pathogenicity-associated genes, and the evidence for its effects on neuroinflammatory diseases.

Keywords *Helicobacter pylori* • Immunomodulation • T cells • Dendritic cells • Multiple sclerosis

Helicobacter pylori

Helicobacter pylori is a very common Gram-negative bacterial pathogen (Fig. 6.1), which colonizes the gastric mucosa of almost half of all people on the planet [40, 59]. The infection is usually acquired in early childhood and persists lifelong unless antibiotic treatment is given [13]. Chronic colonization with *H. pylori* is the leading cause of peptic ulceration and gastric adenocarcinoma. Over 90 % of those with duodenal ulceration and over 70 % of those with gastric ulceration are infected with *H. pylori* [59]. Despite this very strong causal link, these outcomes only occur in a small proportion of those infected. The lifetime risk of peptic ulceration for those infected is just 10 %, and the risk of developing gastric adenocarcinoma is 2–5 % [12, 105, 135]. Gastric adenocarcinoma is the fifth most common malignancy [36] and the third leading cause of cancer-associated deaths worldwide [40]. *H. pylori* was classified as a human carcinogen over 20 years ago [78] and is the biggest modifiable risk factor for the development of gastric adenocarcinoma. The risk is three to six times higher when the infection

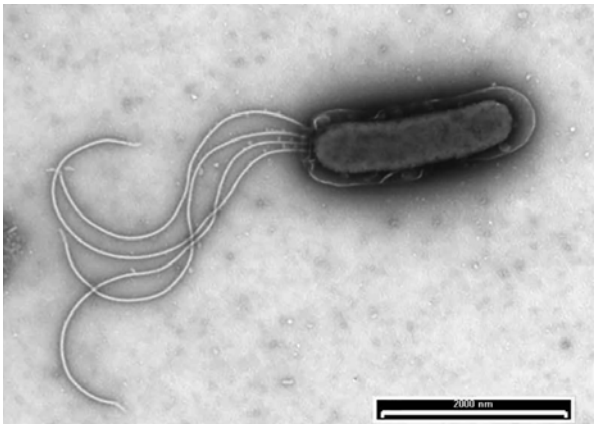

Fig. 6.1 Electron micrograph of a negatively stained preparation of *H. pylori*

is present [36, 40, 59, 130]. Disease is thought to occur due to an interplay of many different factors such as virulence factors expressed by the colonizing strain, host genetics and nature of the immune response, and environmental factors (particularly smoking and diet) [13, 44, 58, 72]. There is also evidence that the infection contributes to increased risk and/or severity of a number of extragastric conditions. These include iron deficiency anaemia, growth retardation in children, and some autoimmune conditions including neuromyelitis optica (NMO) and idiopathic thrombocytopenic purpura (ITP) [147, 171].

The prevalence of *H. pylori* around the world has been declining over the last five decades, and fewer children are now infected [13, 18]. In many developing countries, *H. pylori* remains present in over 80 % of the population, whereas in developed parts of the world, the prevalence is below 20 % overall, and less than 10 % of children are infected. A number of factors are thought to contribute to this, including common antibiotic use in children [105, 135, 186]. Exposure to infectious organisms, particularly during childhood, is thought to be important for the development of a healthy immune system. This was originally referred to as the "hygiene hypothesis" [174], but it has now been renamed the "old friends hypothesis" with realization that modernization diminishes access to many of the necessary immunoregulatory exposures [149]. These include intestinal helminths and gut commensal bacteria, ticks, and soil mycobacteria [8, 106, 117, 149, 150, 183]. *H. pylori* is now emerging as an important member of this group.

Reduced prevalence of the infection is beneficial, preventing peptic ulceration and gastric cancer; however recent evidence suggests that a lack of exposure to *H. pylori* may have adverse consequences. Over the last 60,000 years, human physiology has developed in concert with *H. pylori* in the stomach [13, 87]. Autoimmunity, allergy, asthma, inflammatory bowel disease, and other chronic conditions have become more common as the infection has declined [77, 163]. There are multiple reports of a correlation between *H. pylori* infection and reduced risk of immune and inflammatory diseases, including autoimmune disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and coeliac disease [90, 107, 158, 171], allergic asthma [10, 25], and inflammatory bowel disease [30, 151]. The mechanisms behind many of these associations are thought to involve *H. pylori*-mediated immunomodulation [10].

The Immune Response to *H. pylori*

H. pylori stimulates a strong host response in vivo, which in the first instance involves inflammatory cytokine and chemokine expression by gastric epithelial cells. These factors attract the infiltration of neutrophils, macrophages, dendritic cells (DCs), NK cells, and lymphocytes, and a strong antibody is also elicited [133, 146, 175]. The epithelial barrier interacts with both *H. pylori* and the underlying immune cells. The level and type of the immune response vary, depending on factors such as innate recognition of the bacteria and host genetic differences. Genetic

polymorphisms, such as those in cytokine and Toll-like receptor genes, influence the severity of the inflammatory response, which in turn affects the risk of disease development [54, 103].

Innate Immunity and Inflammation

As with the vast majority of infections, initial detection of *H. pylori* occurs via pattern recognition receptors (PRRs). These include Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which bind specific pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), flagellins, and cell wall peptides [169]. *H. pylori* is unusual as it has evolved mechanisms to minimize PRR activation [157], presumably so that it can maintain persistent colonization of the gastric mucosa. For example, its tetra-acetylated LPS is poorly recognized by TLR4 [45, 116], and the *flaA* gene contains a mutation which dramatically reduces flagellin binding to TLR5 [7]. Despite this, *H. pylori* PAMPs do activate PRRs. TLR2 appears to be the main receptor for LPS [121, 170], but TLR2 is also activated by other components including heat shock protein 60 (HSP60) and *H. pylori* neutrophil-activating protein (HP-NAP) [169]. In addition, interactions of the cytotoxin-associated gene pathogenicity island (*cag* PAI)-encoded type IV secretion system (T4SS) with host epithelial cells result in NOD1 activation and increased pro-inflammatory gene expression [180]. This occurs via transfer of soluble peptidoglycan components into the cytoplasm [185]. Such interactions generally result in increased interleukin 8 (IL-8) expression by epithelial cells and increased IL-6 secretion from dendritic cells and macrophages.

Pro-inflammatory chemokines and cytokines, such as IL-8, IL-1β, tumour necrosis factor alpha (TNFα), IL-6, IL-12, CCL2-5, CCL20, and CXCL1-3, are upregulated in the *H. pylori*-infected gastric mucosa, and the expression of homing receptors is also increased [42, 52, 134, 192, 195]. This leads to the recruitment of immune cells, including neutrophils, macrophages, dendritic cells, NK cells, and lymphocytes; however *H. pylori* has multiple immune evasion strategies [13]. Neutrophils and macrophages attempt to control the infection by phagocytosis; however *H. pylori* prevents the oxidative burst and can survive intracellularly [4, 162]. *H. pylori*-derived arginase also inhibits nitric oxide production [67]. Both M1 and M2 macrophages are present in the infected gastric mucosa [88, 141], and macrophage-derived cytokines have an important influence on the development and balance of damaging and immunomodulatory T helper subset responses [119].

There is a paucity of data on invariant lymphocytic cells (ILCs) and NK cells during *H. pylori* infection; however NKT cells are more abundant in the gastric mucosa [125], and increased numbers of NK cells have also been detected in the peripheral blood [153]. The inflammatory cytokine response of NK cells may be modulated by exposure to *H. pylori* [154].

There are also increased numbers of dendritic cells (DCs) in *H. pylori*-infected gastric tissue from humans [24, 129] and mice [3, 50]. These are $CD11c^+$, indicating

that they are of a myeloid type (mDC) [24, 83, 129]. Oertli et al. [129] showed that mucosal DCs tend to be DC-SIGN$^+$, HLA-DR^{hi}, CD80^{lo}, and CD86^{lo} and have a semi-mature and tolerogenic phenotype. Reduced numbers of pDCs have been found in the peripheral blood of *H. pylori*-infected adults with ITP, a disorder caused by autoreactive antibodies against platelets, but mDC populations were unaffected [156].

Adaptive Immunity

Strong IgG and IgA antibody responses are detected in *H. pylori*-infected individuals [191], and this may contribute to pathogenesis by triggering autoimmunity. The molecular mimicry of host antigens by *H. pylori* elicits an antibody response which reacts with human antigens such as the parietal cell H^+, K^+-ATPase in the gastric mucosa [46]. These autoreactive antibodies are commonly found in the serum of infected patients and may be responsible for increasing local inflammation and tissue damage in the stomach or contribute to extra-gastric autoimmune conditions.

The T-cell response to *H. pylori* infection includes both CD4$^+$ T helper (Th) and CD8$^+$ cytotoxic T cells, but most research has focussed on the Th response. Increased numbers of CD8$^+$ cells are present in the gastric mucosa and peripheral blood of infected humans and the stomachs of *H. pylori*-infected mice [63]. These contribute to *H. pylori* inflammation and disease, possibly by expressing cytokines such as IL-17 [32, 178].

The main Th subsets induced by *H. pylori* infection are pro-inflammatory Th17 and Th1 and anti-inflammatory regulatory T-cell (Treg) populations [5, 56, 140, 148, 165]. Increased numbers of these cell types have been found in the gastric mucosa and peripheral blood of infected donors [42, 172, 187]. Th cells orchestrate the nature of the host response, are thought to be an important contributing factor in determining *H. pylori*-associated disease risk, and have an important impact on *H. pylori*-mediated protection from immune and inflammatory diseases.

Th1 cells primarily secrete interferon-gamma (IFNγ) and TNFα and induce macrophages to secrete further pro-inflammatory mediators and have more bactericidal activity [76, 139]. Th17 cells secrete IL-17A, IL-17 F, IL-21, and IL-22, and these also exert important antibacterial and inflammatory effects including the expression of antimicrobial peptides, stimulation of reactive oxygen and nitrogen species, and augmented chemokine expression, leading to neutrophil recruitment (reviewed by [118, 190]). In *H. pylori*-infected mice, the induction of a Th17 response occurs in conjunction with a Th1 response, leading to more severe gastritis [133, 168]. Release of the cytokine B-cell activating factor of TNF family (BAFF) from macrophages exposed to *H. pylori* plays an important role in the differentiation of Th17 cells [119]. *H. pylori* may be adapted to direct the immune system away from a pro-inflammatory Th1/Th17 response and towards a predominant anti-inflammatory Treg response in order to allow persistence [82]. Peptic ulceration is more frequently found in those with a reduced Treg response [148, 166]. Tregs may act in a bystander

manner by secreting immunosuppressive cytokines such as IL-10 and transforming growth factor beta (TGFβ) to modulate inflammation, or they may act in an antigen-specific manner via a myriad of mechanisms (reviewed in [2]).

H. pylori-Induced Immunomodulation

Immunomodulatory Mechanisms

H. pylori infections are usually established in early childhood [51], when the immune system is developing and there is a bias in favour of immunomodulatory responsiveness. The main *H. pylori*-mediated mechanism being investigated in the research field is the stimulation of Tregs. Increased numbers of Tregs are present in the gastric mucosa and peripheral blood of *H. pylori*-infected patients [42, 101, 143, 148, 187]. The infection is well known to protect against allergic asthma in a mouse model [9]. Infected animals had significantly reduced airway hyperresponsiveness, with lower levels of allergen-specific serum IgE, and pulmonary infiltration of Th2 cells, Th17 cells, and eosinophils. The protective effects were strongest in mice that had been infected as neonates and were conferred by Treg cells. *H. pylori* induces DC differentiation into a tolerogenic type, which promotes the differentiation of naive T cells into Tregs [57, 128, 129].

In addition, expression of the co-stimulatory molecule B7-H1 is upregulated in gastric epithelial cells during *H. pylori* infection. Interaction of T cells with this molecule suppresses T-cell activity [48]. *H. pylori* engagement of dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) also caused reduced pro-inflammatory gene expression [71] and modulation of damaging T helper 1 (Th1) subset responses [22]. It has been shown that oral doses of *H. pylori* DNA could substantially reduce the severity of murine colitis models [75, 102]. This was accompanied by increased expression of IL-10 and reduced expression of IL-17 in the draining lymph nodes and mucosal tissues of these mice. These protective effects were also proposed to be mediated through dendritic cells.

IL-10 and TGFβ are known to be upregulated in the serum and gastric mucosa of infected patients, and low levels correlate with increased disease risk. The cellular source of these suppressive factors is not restricted to T cells, but also includes gastric epithelial cells, B cells, monocytic cells, and DCs [23, 61, 82, 95, 188].

H. pylori *Virulence Factors and Immunomodulatory Molecules*

A number of pro-inflammatory molecules, toxins, and adhesins expressed by *H. pylori* are known to increase the risk of developing gastroduodenal disease via effects on both gastric epithelial cells and immune cells. In addition to their disease-contributing effects, some of these factors are also reported to have potent

immunomodulatory activity. They could therefore play a role in *H. pylori*-mediated protection against autoimmune and inflammatory diseases.

Vacuolating Cytotoxin A (VacA) VacA has multiple effects on epithelial immune cells [43]. This pore-forming toxin permits the passage of anions and small molecules through epithelial cell membranes and generates a great number of vacuoles in cultured epithelial cells [92]. The *vacA* gene is present in almost all *H. pylori* strains, but is polymorphic with three areas of biologically important variation [147]. The signal region and intermediate region determine cytotoxic activity and may be of types s1 or s2 and i1 or i2, respectively. The mid-region determines binding to host cells and may be either m1 or m2. s1/i1/m1 forms of VacA are the most active. Many reports from around the world show strong associations between infection with strains expressing more active VacA types and incidence of peptic ulcer disease or gastric adenocarcinoma [14, 15, 62, 84, 110, 111, 121, 144, 152].

VacA also interacts directly with immune cells, and this is thought to have immunomodulatory consequences. Binding to activated human T cells occurs via the β2 (CD18) integrin receptor subunit [167]. VacA inhibits the activation and proliferation of human B and T lymphocytes [182]. In Jurkat T cells, VacA inhibits IL-2 production and downregulates the expression of IL-2 receptor-α, via inhibition of the nuclear factor of activated T cells (NFAT), and blockade of calcium influx and calcineurin activation of the IL-2 promoter [27, 66, 160]. However, VacA inhibition of proliferation in primary human $CD4^+$ T cells was achieved in an NFAT- and IL-2-independent manner, and acid activation of VacA markedly increased its suppressive potency. This suggests that VacA interferes with T-cell proliferation via multiple mechanisms [176].

VacA may also exert immunosuppressive effects via antigen-presenting cells. Exposure to VacA results in downregulation of MHC class II, inhibition of DC maturation, and a reduced capacity for antigen presentation [86, 114]. Experiments in mice have shown that VacA reprogrammes DCs to a more tolerogenic phenotype, which induces the differentiation of naive T cells into Tregs. This has been implicated as a mechanism for *H. pylori*-mediated protection against asthma and other allergic diseases [10, 128, 157]. Recent data has shown that these effects may be induced via infection or through the administration of purified VacA [57].

Gamma-Glutamyl Transpeptidase (GGT) GGT is a potent virulence factor that causes damage to the gastric epithelium. It stimulates inflammatory responses in gastric epithelial cells, with activation of the nuclear factor kappa B (NF-κB) transcription factor, and the generation of reactive oxygen species. This is thought to lead to DNA damage in the gastric mucosa and thus contribute to carcinogenesis [31, 49, 68]. GGT is an essential factor for *H. pylori* colonization in mice [34], most likely by enabling the bacteria to use extracellular glutamine and glutathione as a source of glutamate [145]. In addition to its contribution to virulence, GGT also has potent immunomodulatory activity [161]. It suppresses T-cell activation, proliferation, and cytokine expression during infection, and, in addition to VacA, it plays a key role in *H. pylori*-mediated protection against allergic asthma in mice [128]. The mechanism behind these effects has recently been shown to involve glutamate deprivation of T cells in the gastric mucosa [193].

Cytotoxin-Associated Pathogenicity Island The best known virulence determinant of *H. pylori* is encoded by the cytotoxin-associated gene pathogenicity island (*cag* PAI) [180]. *cag* PAI-positive strains are more commonly associated with peptic ulceration and gastric adenocarcinoma [137]. It encodes components of a type IV secretion system (T4SS) which delivers CagA into gastric epithelial cells. Upon entry to the cytoplasm, CagA is phosphorylated by Src kinases and activates MAP kinase signalling and NF-κB to induce multiple cellular effects [127]. Cellular interactions with the T4SS pilus by itself also result in activation of NOD1, MAP kinase pathways, and the NF-κB and AP-1 transcription factors [29, 69, 89]. These cascades stimulate disruption of the cell cycle, increased apoptosis, and inflammatory cytokine and chemokine expression, leading to a greater risk of peptic ulcer disease and gastric malignancy [16, 26, 122, 123, 134, 180]. Although the *cag* PAI is linked to increased inflammation and disease risk, there are some reports of CagA and $CagA^+$ strains having immunomodulatory activity. Stronger IL-10 and Treg responses are present in those infected with *cagA*+ strains [74, 148], and these infections also provide stronger protective associations with asthma [33]. CagA-dependent T-cell priming in infected mice is also thought to be important for inducing Treg differentiation [85]. Additionally, it has been shown that CagA can inhibit the mitogen-induced proliferation of human T cells [132].

Outer Inflammatory Protein A (OipA) OipA is also thought to be an important driver of disease, acting by enhancing mucosal inflammation [194, 196, 197]. A recent paper reported that recombinant OipA has a suppressive effect on the maturation of mouse spleen DCs in vitro [181].

***H. pylori* Neutrophil-Activating Protein (HP-NAP)** HP-NAP induces the secretion of IL-12 and IL-23 by neutrophils and monocytes, creating an environment which drives differentiation of T cells down the Th1 and Th17 pathways. It has been shown to modulate Th2 responses in humans and mice [5, 6, 35, 47]. For example, when HP-NAP was administered to mice undergoing allergen sensitization, lung eosinophils and serum IgE levels were reduced, and there were lower concentrations of Th2 cytokines in the lung [35].

Multiple Sclerosis and Its Animal Model, Experimental Autoimmune Encephalomyelitis

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory and neurodegenerative disease of the central nervous system (CNS), which predominantly affects young adults and represents a leading cause of neurological disability in this age group. Other age groups are also affected, from childhood to advanced age [38]. There are two main clinical courses of MS, on the basis of whether disease onset is characterized by an acute attack of neurological dysfunction or not. The most common form at presentation is relapsing-remitting (RR) MS, manifesting as recurrent attacks (relapses) of neurological dysfunction followed by periods of remission. After a variable period of time (typically 10–20 years), this is followed, in about 50 % of patients,

by a gradual progression, with or without superimposed relapses, called secondary progressive (SP) MS. The other main type of clinical course, observed in approximately 15 % of patients, is characterized by progressive neurological dysfunction from the onset and is called primary progressive (PP) MS [100].

Neuroinflammation in MS can involve any part of the CNS, and the most common manifestations are sensory, motor, and visual disturbances, bladder and bowel dysfunction, and balance problems [38]. Neuropathic pain and cognitive disturbances are also quite common and increasingly recognized. Mild to moderate fatigue is also extremely common in MS, and when it is severe, it can be one of the most disabling symptoms. Its mechanisms are poorly understood [79].

The pathogenesis of MS is multifactorial. On the basis of a genetic susceptibility to autoimmunity in general and CNS damage more specifically, environmental factors are thought to trigger damage directed towards CNS myelin [80, 124]. Amongst infectious environmental factors, exposure to herpes viruses during adolescence appears to play an important role. The strongest epidemiological links are with post-childhood exposure to Epstein-Barr virus (EBV; human herpesvirus 4, HHV4) [11]. Importantly, other infections, including early exposure to *H. pylori* may exert a protective effect not only against MS but also other immune-mediated diseases [41, 149, 179]. Noninfectious environmental factors thought to be involved as MS triggers include smoking and low vitamin D [19].

The target of the autoimmune response in MS is CNS myelin, which is produced by oligodendrocytes. Therefore, much study has gone into the identification of specific myelin protein targets. The most abundant protein components of CNS myelin, myelin basic protein (MBP) and proteolipid protein (PLP), are also expressed in the peripheral nervous system (PNS), which is not affected by MS, and are therefore unlikely to be primary targets of the autoimmune response. Myelin oligodendrocyte glycoprotein (MOG), on the other hand, is only expressed in the CNS and could be a more important target of immune-mediated damage. It is possible that once autoreactivity starts against one protein, it can then spread to other antigenic epitopes of the same protein or indeed to other myelin proteins [124, 142]. Every cell type of the immune system, serving the cellular and humoral, the innate and adaptive immune responses, is involved in the orchestration of the inflammatory demyelinating damage. Although the myelin sheath and the oligodendrocyte are considered the main targets of the pathological process, other neural cells are affected by MS. For example, it is the secondary damage to the demyelinated neuronal axons that correlates most closely with chronic loss of brain and spinal cord volume and with the accumulation of irreversible disability in MS [173, 184]. Microglia and astrocytes are also affected, displaying both protective and pathogenic features, as reviewed elsewhere [104, 109]. Experimental autoimmune encephalomyelitis (EAE), a useful albeit imperfect model of MS [39], has provided evidence for the involvement of adaptive and innate immunity in the induction of CNS inflammatory demyelination [138], as well as for the importance of the blood-brain barrier (BBB) in controlling the influx of inflammatory molecules (and consequently cells) from the peripheral circulation to the CNS. EAE has been criticized for the low rate of translational success of treatment from rodent models to human disease. Nevertheless, it has been the source of significant success in the development of some of the most effective treatments available for MS. It is discussed more extensively in recent reviews [17, 177].

Links Between *H. pylori* and Neuromyelitis Optica

Neuromyelitis optica (NMO) is an immune-mediated disorder of the central nervous system (CNS) preferentially affecting the optic nerves and the spinal cord. NMO patients tend to have serum IgG antibodies against the astrocyte water channel aquaporin-4 (AQP4), and this is a useful marker for distinguishing NMO from multiple sclerosis (MS) [20, 131]. Interestingly, AQP4 is also expressed by gastric acid-secreting parietal cells, and a recent study has shown that its expression in the gastric mucosa of mice is influenced by *H. pylori* infection [64, 108]. Patients with positive AQP4 serology and NMO also tend to have antibodies that react with gastric parietal cells [81].

Several papers have indicated a positive association between *H. pylori* infection and NMO. Li et al. [93] reported that rates of *H. pylori* serology were higher amongst AQP4 antibody-positive NMO patients. A second study also determined that *H. pylori* was significantly more common amongst NMO patients compared with healthy controls [98]. A further study of 116 Japanese NMO patients and 367 healthy controls reported that *H. pylori* was significantly more common amongst the AQP4 antibody-positive NMO group than the healthy controls. There was no difference between the controls and the AQP4 seronegative NMO group, however, and it was concluded that *H. pylori* infection is a risk factor for AQP4 antibody-positive NMO [200]. There has recently been a published case report of a patient presenting simultaneously with AQP4 seropositive NMO and ITP (an autoimmune platelet disorder associated with *H. pylori* infection) [112]. After eradication of *H. pylori*, the titre of AQP4 IgG was reduced, platelet counts returned to normal levels, and the symptoms resolved almost completely. In a further paper, it was shown that the *H. pylori* virulence factor HP-NAP may be associated with pathology and neural damage in AQP4 antibody-positive NMO, as there was a significant positive correlation between anti-HP-NAP serology, concentrations of the inflammatory marker myeloperoxidase in serum, and disability scale scores [93]. These papers all indicate a role for *H. pylori* in NMO. It therefore seems likely this association could be driven via induction of an autoreactive antibody response in the gastric mucosa.

Links Between *H. pylori* and Multiple Sclerosis

Epidemiology

Several epidemiological studies (Table 6.1) have reported a significantly lower prevalence of *H. pylori* infection amongst MS patients [41, 60, 94, 113, 189, 199]. Two case-control studies found that amongst MS patients, those with *H. pylori* had reduced levels of neurological disability [94, 113]. Others have failed to find any association between *H. pylori* infection and MS, however, and perhaps this is because of differences in the classifications of MS and NMO between studies, the methods used to determine *H. pylori* status, or small group sizes [65].

Factors such as gender, age, and social class have a strong influence on *H. pylori* infection rates, and it is more common in older people, in males, and in those of

Table 6.1 Summary of epidemiological studies on *H. pylori* and multiple sclerosis

Evidence for a negative association		Reference
Study of 90 Polish MS patients	18.9 % *H. pylori* seropositivity, much lower than the general prevalence rate in Poland	[189]
105 Japanese MS patients (divided into 52 with optico-spinal MS (OSMS) and 53 with conventional MS (CMS)) and 85 healthy controls	*H. pylori* seropositivity was lower in CMS patients compared with OSMS ($p=0.0019$) and healthy controls ($p=0.018$). Patients with CMS had a significantly lower disability score if *H. pylori* positive ($p=0.03$)	[94]
Case-control study of 163 Iranian MS patients and 150 age- and sex-matched healthy controls	*H. pylori* positive serology in 54 % of MS patients compared with 73 % of controls ($p<0.001$). Significantly reduced scores amongst the *H. pylori*-infected patients on the Expanded Disability Status Scale ($p=0.017$)	[113]
Study of 90 Japanese MS patients (none with NMO) and 177 healthy controls	Significantly reduced number of *H. pylori* seropositive patients amongst the MS group ($p=0.045$)	[199]
71 MS patients from the UK (48 with relapsing-remitting MS, 19 with secondary progressive MS, and 4 with primary progressive MS) and 42 age- and gender-matched healthy controls	21 % of MS patients were *H. pylori* seropositive, compared with 42.9 % of healthy controls ($p=0.018$)	[41]
550 Australian MS patients and 299 age- and gender-matched healthy controls	*H. pylori* seropositivity lower amongst MS patients compared to controls, but only statistically significant amongst females ($p=0.027$). *H. pylori*-infected females had lower disability scores than the uninfected females ($p=0.049$); however the reverse was true amongst the males ($p=0.025$). No association between *H. pylori* status and relapse rate	[60]
No evidence of an association		
145 Japanese MS patients and 367 healthy controls	No differences in anti-*H. pylori* antibody positivity between the groups	[201]
135 AQP4 antibody-negative Japanese MS patients (52 with OSMS and 85 with CMS) and 85 healthy controls	No significant difference in *H. pylori* seropositivity between the groups	[93]
Evidence for a positive association		
29 MS patients and 25 anaemic controls	Significantly increased proportion of *H. pylori*-infected patients amongst the MS group ($p=0.007$)	[65]

lower socioeconomic status [105]. In contrast, MS is more common in females of a higher socioeconomic status [115]. The recently published study by Fabis Pedrini et al. [60] was based on large groups of MS patients and age- and gender-matched healthy controls. They found that *H. pylori* seropositivity was lower amongst the MS patients; however this was only statistically significant amongst the females. *H. pylori*-infected female MS patients had lower disability scores than the uninfected female MS patients, but surprisingly the reverse was true amongst the males. Differences in the type of MS also have an impact on studies aiming to identify associations with *H. pylori* status. Li et al. [94] found that *H. pylori* seropositivity was lower amongst conventional MS (CMS) patients compared to those with opticospinal MS (OSMS), which is a variant of MS with similar clinical features to NMO and common in Asian populations. This illustrates the need for studies to be carefully controlled for confounding influences and bias.

To date, there is little mechanistic evidence of *H. pylori*-mediated protection against MS, and it is possible that presence of the infection could merely be a marker for other co-exposures which actually drive the protective effects. Unfortunately as yet there have been no published case studies on the effects of *H. pylori* eradication therapy in MS patients; however such combinations of antibiotics would also have an impact on the entire bacterial microflora. This would make it difficult to assess the role of *H. pylori* in particular. It would be helpful if it could be shown, as in asthma research, that associations are stronger in people infected with a particular subset of *H. pylori* strain types [33]. The best way to prove whether *H. pylori* is protective against MS would be to administer the infection or its components to patients. It has only recently become possible to deliberately infect healthy volunteers with *H. pylori*, and adverse effects were observed [1, 70]. This makes it an unlikely strategy to inhibit the development of MS or reduce disease progression. One article has called for the development of *H. pylori* nanoparticles as a treatment for MS [136]. This could be a useful alternative, especially if the protective bacterial components can be identified. Many of the protective effects attributed to *H. pylori* could require infection from an early age or even throughout life. This also complicates strategies to understand the mechanisms and harness them for therapies.

Experiments with Animal Models

Only one animal study on *H. pylori* and its impact on a model of MS has been reported so far. Our group showed that prior *H. pylori* infection of mice inhibited the severity of experimental autoimmune encephalomyelitis (EAE) [41]. This is the most commonly used model for human MS [39]. EAE was induced by immunization with the myelin oligodendrocyte glycoprotein (MOG) peptide $MOG_{35–55}$ in a strong adjuvant formulation, leading to an autoimmune response that mimics MS [39, 177]. It has been shown that injection of mice with heat killed *H. pylori* bacteria, and Freund's incomplete adjuvant, however, was not sufficient to trigger EAE [28]. Over three independent EAE experiments, we found that there were

significantly reduced clinical scores in mice previously infected with *H. pylori,* compared to groups that were administered placebo doses. The average maximal scores were also lower; however there was no delay in the onset of EAE [41]. Effects of the infection on the severity of EAE were therefore only moderate, especially when this is compared to other bacterial treatments such as daily administration of the *Bacteroides fragilis* PSA (polysaccharide) which protected against the development of EAE [126]. The impact of *H. pylori* on the T-cell responses in EAE mice, however, was very marked.

Firstly, MOG peptide-specific proliferation of splenic T cell from infected EAE mice was significantly reduced by threefold in comparison with cells from uninfected EAE mice [41]. Similar findings were shown in the response of spleen cells to polyclonal T-cell activation. Since EAE is characterized by infiltration of $CD4^+$ and $CD8^+$ cells into the CNS [120], at the peak in EAE severity, we also investigated whether there were differences in the size of these populations in the spinal cords of *H. pylori*-infected and *H. pylori*-uninfected mice [41]. The populations were indeed reduced, by 4.5-fold and 2.5-fold, respectively, and we consider that this is probably responsible for the difference in EAE clinical scores. Both $CD4^+$ and $CD8^+$ cells play an important role in EAE and MS. $CD8^+$ cells cause inflammatory lesions in the optic nerve, brain, and spinal cord, with focal loss of oligodendrocytes and axonal damage [159].

We then went on to investigate differences in the frequencies of Th1 and Th17 cells in the CNS, hypothesizing that these would also be reduced since they play a major role in EAE pathogenesis [99]. The proportion of Th1 cells (identified as T-bet$^+$ and IFNγ^+) amongst the $CD4^+$ population in the infected EAE mice was half that of the uninfected EAE group. There were similar reductions in Th17 cells (RORγt$^+$ and IL-17$^+$). Differences in Th1 and Th17 populations in the spleen were much larger, however. Th1 cells were reduced by 31-fold in infected EAE mice and Th17 cells by 11-fold.

The balance between Th1/Th17 and Treg subsets is important in MS development and progression [53, 164]. Because of this and the wealth of data on *H. pylori*-mediated immunomodulation (see section "*H. pylori*-Induced Immunomodulation" above), we therefore hypothesized that there would be increased frequencies of $Foxp3^+$ $CD4^+$ cells in infected EAE mice. Surprisingly no differences were observed, either in the spinal cord or the spleen. Unfortunately because we limited our quantification of Tregs to $Foxp3^+$ cells, we must now begin to examine other Treg and immunomodulatory cell populations. The most likely mechanism of protection is via IL-10, since *H. pylori*-induced Tregs tend to act by secretion of this suppressive factor [10, 148]. It has recently been shown that a subset of FoxA1$^+$, Foxp3$^-$ Tregs are protective against EAE. This IFNβ-responsive cell type is present in MS patients and has an impact on the effectiveness of IFNβ therapy [97]. It is as yet unknown whether these cells are influenced by *H. pylori* infection status.

Another possibility is that the infection may inhibit EAE by altering the expression of chemokine receptors and integrins by T effector or regulatory T cells, leading to reduced numbers of T cells entering the CNS. We have previously reported that *H. pylori* infection results in increased numbers of human peripheral blood

Tregs expressing the chemokine receptor CCR6 and that there are high concentrations of CCL20 (the ligand for CCR6) in the infected gastric mucosa [42]. CCR6 is also thought to play an important role in moderating the balance between Tregs and Th17 cells [37], and it has an impact on EAE [55, 96]. It is therefore possible that the trafficking of $CCR6^+$ cells is diverted to the inflamed stomach. The peak of EAE severity correlates with DC recruitment to the CNS [155]. Since *H. pylori* infection reduced the peak EAE clinical scores, the involvement of DCs in protection should also be investigated in the future.

H. pylori may merely be acting as a marker for other protective co-exposures and infections. Evidence using the Mongolian gerbil infection model has shown that *H. pylori* infection alters the microbiota of the gastrointestinal tract [73, 198]. Since the gut microbiota is known to have an impact on EAE [21, 91], it is possible that the protective effects of *H. pylori* are not mediated directly.

Conclusions

Epidemiological and serological evidence points to an inverse association between *H. pylori* infection and MS. This is supported by studies in the experimental model, EAE, in which exposure to *H. pylori* reduces the severity of the autoimmune T cell-mediated central nervous system inflammation, with a reduction in the production of pro-inflammatory cytokines. The exact mechanisms of this protection are unclear and are currently being investigated, but, based on evidence from previous studies, immunoregulatory networks activated by the infection with *H. pylori* are likely to play an important role. On the other hand, *H. pylori* may show a positive association with another neuroinflammatory disease, NMO. This reflects the distinct underlying immunopathological mechanisms of MS and NMO. The further elucidation of the role of *H. pylori* in the pathogenesis of neuroinflammatory diseases is likely to improve our insight both into the potential for immunomodulatory strategies of these diseases and into the complex effects of *H. pylori* on the immune system.

References

1. Aebischer T, Bumann D, Epple HJ, Metzger W, Schneider T, Cherepnev G, Walduck AK, Kunkel D, Moos V, Loddenkemper C, Jiadze I, Panasyuk M, Stolte M, Graham DY, Zeitz M, Meyer TF. Correlation of T cell response and bacterial clearance in human volunteers challenged with *Helicobacter pylori* revealed by randomised controlled vaccination with Ty21a-based *Salmonella* vaccines. Gut. 2008;57(8):1065–72. doi:10.1136/gut.2007.145839.
2. Ai TL, Solomon BD, Hsieh CS. T-cell selection and intestinal homeostasis. Immunol Rev. 2014;259(1):60–74. doi:10.1111/imr.12171.
3. Algood HM, Gallo-Romero J, Wilson KT, Peek Jr RM, Cover TL. Host response to *Helicobacter pylori* infection before initiation of the adaptive immune response. FEMS Immunol Med Microbiol. 2007;51(3):577–86. doi:10.1111/j.1574-695X.2007.00338.x.

4. Allen LA, Beecher BR, Lynch JT, Rohner OV, Wittine LM. *Helicobacter pylori* disrupts NADPH oxidase targeting in human neutrophils to induce extracellular superoxide release. J Immunol. 2005;174(6):3658–67.
5. Amedei A, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G, de Bernard M. The neutrophil-activating protein of *Helicobacter pylori* promotes Th1 immune responses. J Clin Invest. 2006;116(4):1092–101. doi:10.1172/JCI27177.
6. Amedei A, Codolo G, Del Prete G, de Bernard M, D'Elios MM. The effect of *Helicobacter pylori* on asthma and allergy. J Asthma Allergy. 2010;3:139–47. doi:10.2147/JAA.S8971.
7. Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, Aderem A. Evasion of toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci U S A. 2005;102(26):9247–52. doi:10.1073/pnas.0502040102.
8. Araujo MI, Hoppe BS, Medeiros Jr M, Carvalho EM. *Schistosoma mansoni* infection modulates the immune response against allergic and auto-immune diseases. Mem Inst Oswaldo Cruz. 2004;99(5 Suppl 1):27–32.
9. Arnold IC, Dehzad N, Reuter S, Martin H, Becher B, Taube C, Muller A. *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. J Clin Invest. 2011;121(8):3088–3093. doi:45041 [pii] 10.1172/JCI45041.
10. Arnold IC, Hitzler I, Muller A. The immunomodulatory properties of *Helicobacter pylori* confer protection against allergic and chronic inflammatory disorders. Front Cell Infect Microbiol. 2012;2:10. doi:10.3389/fcimb.2012.00010.
11. Ascherio A. Environmental factors in multiple sclerosis. Expert Rev Neurother. 2013;13(12 Suppl):3–9. doi:10.1586/14737175.2013.865866.
12. Atherton JC. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. Annu Rev Pathol. 2006;1:63–96.
13. Atherton JC, Blaser MJ. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. J Clin Invest. 2009;119(9):2475–87. doi:10.1172/JCI38605.
14. Atherton JC, Cao P, Peek Jr RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J Biol Chem. 1995;270(30):17771–7.
15. Atherton JC, Peek Jr RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology. 1997;112(1):92–9.
16. Backert S, Selbach M. Role of type IV secretion in *Helicobacter pylori* pathogenesis. Cell Microbiol. 2008;10(8):1573–81. doi:10.1111/j.1462-5822.2008.01156.x.
17. Baker D, Amor S. Mouse models of multiple sclerosis: lost in translation? Curr Pharm Des. 2015;21(18):2440–52.
18. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. J Infect Dis. 1993;168(1):219–21.
19. Belbasis L, Bellou V, Evangelou E, Ioannidis JP, Tzoulaki I. Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses. Lancet Neurol. 2015;14(3):263–73. doi:10.1016/S1474-4422(14)70267-4.
20. Bennett J, de Seze J, Lana-Peixoto M, Palace J, Waldman A, Schippling S, Tenembaum S, Banwell B, Greenberg B, Levy M, Fujihara K, Chan K, Kim H, Asgari N, Sato D, Saiz A, Wuerfel J, Zimmermann H, Green A, Villoslada P, Paul F, with the G-I, Br. Neuromyelitis optica and multiple sclerosis: seeing differences through optical coherence tomography. Mult Scler. 2015;21:678–88. doi:10.1177/1352458514567216.
21. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, Wekerle H, Krishnamoorthy G. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature. 2011;479(7374):538–41. doi:10.1038/nature10554.
22. Bergman MP, Engering A, Smits HH, van Vliet SJ, van Bodegraven AA, Wirth HP, Kapsenberg ML, Vandenbroucke-Grauls CM, van Kooyk Y, Appelmelk BJ. *Helicobacter pylori* modulates the T helper cell 1/T helper cell 2 balance through phase-variable interaction between lipopolysaccharide and DC-SIGN. J Exp Med. 2004;200(8):979–90.

23. Beswick EJ, Pinchuk IV, Earley RB, Schmitt DA, Reyes VE. Role of gastric epithelial cell-derived transforming growth factor beta in reduced CD4+ T cell proliferation and development of regulatory T cells during *Helicobacter pylori* infection. Infect Immun. 2011;79(7):2737–45. doi:10.1128/IAI.01146-10.
24. Bimczok D, Clements RH, Waites KB, Novak L, Eckhoff DE, Mannon PJ, Smythies LE. Human primary gastric dendritic cells induce a Th1 response to *H. pylori*. Mucosal Immunol. 2010;3(3):260–9. doi:10.1038/mi.2010.10.
25. Blaser MJ, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? Gut. 2008;57(5):561–7.
26. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 1995;55(10):2111–5.
27. Boncristiano M, Paccani SR, Barone S, Ulivieri C, Patrussi L, Ilver D, Amedei A, D'Elios MM, Telford JL, Baldari CT. The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms. J Exp Med. 2003;198(12):1887–97.
28. Boziki M, Grigoriadis N, Deretzi G, Lagoudaki R, Lourbopoulos A, Panayotopoulou E, Sgouras D, Tascos N, Kountouras J. *Helicobacter pylori* immunomodulatory properties in a mouse model of multiple sclerosis. Immuno-Gastroenterol. 2012;1(1):34–9. doi:10.7178/ig.1.1.8.
29. Brandt S, Kwok T, Hartig R, Konig W, Backert S. NF-kappaB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. Proc Natl Acad Sci U S A. 2005;102(26):9300–5.
30. Budzynski J, Klopocka M. Brain-gut axis in the pathogenesis of *Helicobacter pylori* infection. World J Gastroenterol. 2014;20(18):5212–25. doi:10.3748/wjg.v20.i18.5212.
31. Busiello I, Acquaviva R, Di Popolo A, Blanchard TG, Ricci V, Romano M, Zarrilli R. *Helicobacter pylori* gamma-glutamyltranspeptidase upregulates COX-2 and EGF-related peptide expression in human gastric cells. Cell Microbiol. 2004;6(3):255–67.
32. Caruso R, Fina D, Paoluzi OA, Del Vecchio Blanco G, Stolfi C, Rizzo A, Caprioli F, Sarra M, Andrei F, Fantini MC, MacDonald TT, Pallone F, Monteleone G. IL-23-mediated regulation of IL-17 production in *Helicobacter pylori*-infected gastric mucosa. Eur J Immunol. 2008; 38(2):470–8.
33. Chen Y, Blaser MJ. Inverse associations of *Helicobacter pylori* with asthma and allergy. Arch Intern Med. 2007;167(8):821–7.
34. Chevalier C, Thiberge JM, Ferrero RL, Labigne A. Essential role of *Helicobacter pylori* gamma-glutamyltranspeptidase for the colonization of the gastric mucosa of mice. Mol Microbiol. 1999;31(5):1359–72.
35. Codolo G, Mazzi P, Amedei A, Del Prete G, Berton G, D'Elios MM, de Bernard M. The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. Cell Microbiol. 2008;10(11):2355–63. CMI1217 [pii]. doi:10.1111/j.1462-5822.2008.01217.x.
36. Colquhoun A, Arnold M, Ferlay J, Goodman KJ, Forman D, Soerjomataram I. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut. 2015. doi:10.1136/gutjnl-2014-308915.
37. Comerford I, Bunting M, Fenix K, Haylock-Jacobs S, Litchfield W, Harata-Lee Y, Turvey M, Brazzatti J, Gregor C, Nguyen P, Kara E, McColl SR. An immune paradox: how can the same chemokine axis regulate both immune tolerance and activation?: CCR6/CCL20: a chemokine axis balancing immunological tolerance and inflammation in autoimmune disease. Bioessays. 2010;32(12):1067–76. doi:10.1002/bies.201000063.
38. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502–17. doi:10.1016/S0140-6736(08)61620-7.
39. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011;164(4):1079–106. doi:10.1111/j.1476-5381.2011.01302.x.
40. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. *H. pylori* infection and gastric cancer: state of the art (review). Int J Oncol. 2013;42(1):5–18. doi:10.3892/ijo.2012.1701.

41. Cook KW, Crooks J, Hussain K, O'Brien K, Braitch M, Kareen H, Constantinescu CS, Robinson K, Gran B. *Helicobacter pylori* infection reduces disease severity in an experimental model of multiple sclerosis. Front Microbiol. 2015;8:52.
42. Cook KW, Letley DP, Ingram RJ, Staples E, Skjoldmose H, Atherton JC, Robinson K. CCL20/CCR6-mediated migration of regulatory T cells to the *Helicobacter pylori*-infected human gastric mucosa. Gut. 2014;63(10):1550–9. doi:10.1136/gutjnl-2013-306253.
43. Cover TL, Blanke SR. *Helicobacter pylori* VacA, a paradigm for toxin multifunctionality. Nat Rev Microbiol. 2005;3(4):320–32. doi:10.1038/nrmicro1095.
44. Cover TL, Peek Jr RM. Diet, microbial virulence, and *Helicobacter pylori*-induced gastric cancer. Gut Microbes. 2013;4(6):482–93. doi:10.4161/gmic.26262.
45. Cullen TW, Giles DK, Wolf LN, Ecobichon C, Boneca IG, Trent MS. *Helicobacter pylori* versus the host: remodeling of the bacterial outer membrane is required for survival in the gastric mucosa. PLoS Pathog. 2011;7(12), e1002454. doi:10.1371/journal.ppat.1002454.
46. D'Elios MM, Appelmelk BJ, Amedei A, Bergman MP, Del Prete G. Gastric autoimmunity: the role of *Helicobacter pylori* and molecular mimicry. Trends Mol Med. 2004;10(7):316–23.
47. D'Elios MM, Codolo G, Amedei A, Mazzi P, Berton G, Zanotti G, Del Prete G, de Bernard M. *Helicobacter pylori*, asthma and allergy. FEMS Immunol Med Microbiol. 2009;56(1):1–8.
48. Das S, Suarez G, Beswick EJ, Sierra JC, Graham DY, Reyes VE. Expression of B7-H1 on gastric epithelial cells: its potential role in regulating T cells during *Helicobacter pylori* infection. J Immunol. 2006;176(5):3000–9.
49. Djavaheri-Mergny M, Accaoui MJ, Rouillard D, Wietzerbin J. Gamma-glutamyl transpeptidase activity mediates NF-kappaB activation through lipid peroxidation in human leukemia U937 cells. Mol Cell Biochem. 2002;232(1–2):103–11.
50. Drakes ML, Czinn SJ, Blanchard TG. Regulation of murine dendritic cell immune responses by *Helicobacter felis* antigen. Infect Immun. 2006;74(8):4624–33. doi:10.1128/IAI.00289-06.
51. Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clin Microbiol Rev. 1997;10(4):720–41.
52. Eck M, Schmausser B, Scheller K, Toksoy A, Kraus M, Menzel T, Muller-Hermelink HK, Gillitzer R. CXC chemokines Gro(alpha)/IL-8 and IP-10/MIG in *Helicobacter pylori* gastritis. Clin Exp Immunol. 2000;122(2):192–9.
53. Edstrom M, Mellergard J, Mjosberg J, Jenmalm M, Vrethem M, Press R, Dahle C, Ernerudh J. Transcriptional characteristics of CD4+ T cells in multiple sclerosis: relative lack of suppressive populations in blood. Mult Scler. 2011;17(1):57–66. doi:10.1177/1352458510381256.
54. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni Jr JF, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology. 2003;124(5):1193–201.
55. Elhofy A, Depaolo RW, Lira SA, Lukacs NW, Karpus WJ. Mice deficient for CCR6 fail to control chronic experimental autoimmune encephalomyelitis. J Neuroimmunol. 2009;213(1–2):91–9. doi:10.1016/j.jneuroim.2009.05.011.
56. Enarsson K, Lundgren A, Kindlund B, Hermansson M, Roncador G, Banham AH, Lundin BS, Quiding-Jarbrink M. Function and recruitment of mucosal regulatory T cells in human chronic *Helicobacter pylori* infection and gastric adenocarcinoma. Clin Immunol. 2006;121(3):358–68.
57. Engler DB, Reuter S, van Wijck Y, Urban S, Kyburz A, Maxeiner J, Martin H, Yogev N, Waisman A, Gerhard M, Cover TL, Taube C, Muller A. Effective treatment of allergic airway inflammation with *Helicobacter pylori* immunomodulators requires BATF3-dependent dendritic cells and IL-10. Proc Natl Acad Sci U S A. 2014;111(32):11810–5. doi:10.1073/pnas.1410579111.
58. Epplein M, Zheng W, Li H, Peek Jr RM, Correa P, Gao J, Michel A, Pawlita M, Cai Q, Xiang YB, Shu XO. Diet, *Helicobacter pylori* strain-specific infection, and gastric cancer risk among Chinese men. Nutr Cancer. 2014;66(4):550–7. doi:10.1080/01635581.2014.894096.

59. Ernst PB, Gold BD. The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol. 2000;54:615–40. doi: 10.1146/annurev.micro.54.1.615.
60. Fabis Pedrini MJ, Seewann A, Bennett KA, Wood AJ, James I, Burton J, Marshall BJ, Carroll WM, Kermode AG. *Helicobacter pylori* infection as a protective factor against multiple sclerosis risk in females. J Neurol Neurosurg Psychiatry. 2015. doi:10.1136/jnnp-2014-309495.
61. Fehlings M, Drobbe L, Moos V, Renner Viveros P, Hagen J, Beigier-Bompadre M, Pang E, Belogolova E, Churin Y, Schneider T, Meyer TF, Aebischer T, Ignatius R. Comparative analysis of the interaction of *Helicobacter pylori* with human dendritic cells, macrophages, and monocytes. Infect Immun. 2012;80(8):2724–34. doi:10.1128/IAI.00381-12.
62. Figueiredo C, Van Doorn LJ, Nogueira C, Soares JM, Pinho C, Figueira P, Quint WG, Carneiro F. *Helicobacter pylori* genotypes are associated with clinical outcome in Portuguese patients and show a high prevalence of infections with multiple strains. Scand J Gastroenterol. 2001;36(2):128–35.
63. Figueiredo Soares T, Aguiar Rocha G, Camargos Rocha AM, Correa-Oliveira R, Martins-Filho OA, Teles Carvalho AS, Souto Bittencourt PF, Afonso Oliveira C, Ferreira Nogueira AM, Alvares Cabral MM, Caetano Faria AM, Queiroz DM. Differences in peripheral blood lymphocyte phenotypes between *Helicobacter pylori*-positive children and adults with duodenal ulcer. Clin Microbiol Infect. 2007;13(11):1083–8.
64. Fukuhara S, Matsuzaki J, Tsugawa H, Masaoka T, Miyoshi S, Mori H, Fukushima Y, Yasui M, Kanai T, Suzuki H. Mucosal expression of aquaporin-4 in the stomach of histamine type 2 receptor knockout mice and *Helicobacter pylori*-infected mice. J Gastroenterol Hepatol. 2014;29 Suppl 4:53–9. doi:10.1111/jgh.12771.
65. Gavalas E, Kountouras J, Deretzi G, Boziki M, Grigoriadis N, Zavos C, Venizelos I. *Helicobacter pylori* and multiple sclerosis. J Neuroimmunol. 2007;188(1–2):187–9; author reply 190. doi:10.1016/j.jneuroim.2007.06.007.
66. Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R. *Helicobacter pylori* vacuolating cytotoxin inhibits T lymphocyte activation. Science. 2003;301(5636):1099–102.
67. Gobert AP, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, Mobley HL, Wilson KT. *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. Proc Natl Acad Sci U S A. 2001;98(24):13844–9.
68. Gong M, Ling SS, Lui SY, Yeoh KG, Ho B. *Helicobacter pylori* gamma-glutamyl transpeptidase is a pathogenic factor in the development of peptic ulcer disease. Gastroenterology. 2010;139(2):564–73. doi:10.1053/j.gastro.2010.03.050.
69. Gorrell RJ, Guan J, Xin Y, Tafreshi MA, Hutton ML, McGuckin MA, Ferrero RL, Kwok T. A novel NOD1- and CagA-independent pathway of interleukin-8 induction mediated by the *Helicobacter pylori* type IV secretion system. Cell Microbiol. 2013;15(4):554–70. doi:10.1111/cmi.12055.
70. Graham DY, Opekun AR, Osato MS, El-Zimaity HM, Lee CK, Yamaoka Y, Qureshi WA, Cadoz M, Monath TP. Challenge model for *Helicobacter pylori* infection in human volunteers. Gut. 2004;53(9):1235–43. doi:10.1136/gut.2003.037499.
71. Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Geijtenbeek TB. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to *Mycobacterium tuberculosis*, HIV-1 and *Helicobacter pylori*. Nat Immunol. 2009;10(10):1081–8. doi: 10.1038/ni.1778.
72. Hardbower DM, Peek Jr RM, Wilson KT. At the bench: *Helicobacter pylori*, dysregulated host responses, DNA damage, and gastric cancer. J Leukoc Biol. 2014;96(2):201–12. doi:10.1189/jlb.4BT0214-099R.
73. Heimesaat MM, Fischer A, Plickert R, Wiedemann T, Loddenkemper C, Gobel UB, Bereswill S, Rieder G. *Helicobacter pylori* induced gastric immunopathology is associated with distinct microbiota changes in the large intestines of long-term infected Mongolian gerbils. PLoS One. 2014;9(6), e100362. doi:10.1371/journal.pone.0100362.
74. Hida N, Shimoyama Jr T, Neville P, Dixon MF, Axon AT, Shimoyama Sr T, Crabtree JE. Increased expression of IL-10 and IL-12 (p40) mRNA in *Helicobacter pylori* infected

gastric mucosa: relation to bacterial *cag* status and peptic ulceration. J Clin Pathol. 1999; 52(9):658–64.
75. Higgins PD, Johnson LA, Luther J, Zhang M, Sauder KL, Blanco LP, Kao JY. Prior *Helicobacter pylori* infection ameliorates *Salmonella typhimurium*-induced colitis: mucosal crosstalk between stomach and distal intestine. Inflamm Bowel Dis. 2011;17(6):1398–408. doi:10.1002/ibd.21489.
76. Hoeksema MA, Scicluna BP, Boshuizen MC, van der Velden S, Neele AE, Van den Bossche J, Matlung HL, van den Berg TK, Goossens P, de Winther MP. IFN-gamma priming of macrophages represses a part of the inflammatory program and attenuates neutrophil recruitment. J Immunol. 2015. doi:10.4049/jimmunol.1402077.
77. Hughes AM, Lucas RM, McMichael AJ, Dwyer T, Pender MP, van der Mei I, Taylor BV, Valery P, Chapman C, Coulthard A, Dear K, Kilpatrick TJ, Williams D, Ponsonby AL. Early-life hygiene-related factors affect risk of central nervous system demyelination and asthma differentially. Clin Exp Immunol. 2013;172(3):466–74. doi:10.1111/cei.12077.
78. IARC. Schistosomes, liver flukes and *Helicobacter pylori*, vol. 61. Lyon: IARC Monogr Eval Carcinog Risks Hum. IARC; 1994.
79. Induruwa I, Constantinescu CS, Gran B. Fatigue in multiple sclerosis – a brief review. J Neurol Sci. 2012;323(1–2):9–15. doi:10.1016/j.jns.2012.08.007.
80. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, Dilthey A, Su Z, Freeman C, Hunt SE, Edkins S, Gray E, Booth DR, Potter SC, Goris A, Band G, Oturai AB, Strange A, Saarela J, Bellenguez C, Fontaine B, Gillman M, Hemmer B, Gwilliam R, Zipp F, Jayakumar A, Martin R, Leslie S, Hawkins S, Giannoulatou E, D'Alfonso S, Blackburn H, Martinelli Boneschi F, Liddle J, Harbo HF, Perez ML, Spurkland A, Waller MJ, Mycko MP, Ricketts M, Comabella M, Hammond N, Kockum I, McCann OT, Ban M, Whittaker P, Kemppinen A, Weston P, Hawkins C, Widaa S, Zajicek J, Dronov S, Robertson N, Bumpstead SJ, Barcellos LF, Ravindrarajah R, Abraham R, Alfredsson L, Ardlie K, Aubin C, Baker A, Baker K, Baranzini SE, Bergamaschi L, Bergamaschi R, Bernstein A, Berthele A, Boggild M, Bradfield JP, Brassat D, Broadley SA, Buck D, Butzkueven H, Capra R, Carroll WM, Cavalla P, Celius EG, Cepok S, Chiavacci R, Clerget-Darpoux F, Clysters K, Comi G, Cossburn M, Cournu-Rebeix I, Cox MB, Cozen W, Cree BA, Cross AH, Cusi D, Daly MJ, Davis E, de Bakker PI, Debouverie M, D'Hooghe MB, Dixon K, Dobosi R, Dubois B, Ellinghaus D, Elovaara I, Esposito F, Fontenille C, Foote S, Franke A, Galimberti D, Ghezzi A, Glessner J, Gomez R, Gout O, Graham C, Grant SF, Guerini FR, Hakonarson H, Hall P, Hamsten A, Hartung HP, Heard RN, Heath S, Hobart J, Hoshi M, Infante-Duarte C, Ingram G, Ingram W, Islam T, Jagodic M, Kabesch M, Kermode AG, Kilpatrick TJ, Kim C, Klopp N, Koivisto K, Larsson M, Lathrop M, Lechner-Scott JS, Leone MA, Leppa V, Liljedahl U, Bomfim IL, Lincoln RR, Link J, Liu J, Lorentzen AR, Lupoli S, Macciardi F, Mack T, Marriott M, Martinelli V, Mason D, McCauley JL, Mentch F, Mero IL, Mihalova T, Montalban X, Mottershead J, Myhr KM, Naldi P, Ollier W, Page A, Palotie A, Pelletier J, Piccio L, Pickersgill T, Piehl F, Pobywajlo S, Quach HL, Ramsay PP, Reunanen M, Reynolds R, Rioux JD, Rodegher M, Roesner S, Rubio JP, Ruckert IM, Salvetti M, Salvi E, Santaniello A, Schaefer CA, Schreiber S, Schulze C, Scott RJ, Sellebjerg F, Selmaj KW, Sexton D, Shen L, Simms-Acuna B, Skidmore S, Sleiman PM, Smestad C, Sorensen PS, Sondergaard HB, Stankovich J, Strange RC, Sulonen AM, Sundqvist E, Syvanen AC, Taddeo F, Taylor B, Blackwell JM, Tienari P, Bramon E, Tourbah A, Brown MA, Tronczynska E, Casas JP, Tubridy N, Corvin A, Vickery J, Jankowski J, Villoslada P, Markus HS, Wang K, Mathew CG, Wason J, Palmer CN, Wichmann HE, Plomin R, Willoughby E, Rautanen A, Winkelmann J, Wittig M, Trembath RC, Yaouanq J, Viswanathan AC, Zhang H, Wood NW, Zuvich R, Deloukas P, Langford C, Duncanson A, Oksenberg JR, Pericak-Vance MA, Haines JL, Olsson T, Hillert J, Ivinson AJ, De Jager PL, Peltonen L, Stewart GJ, Hafler DA, Hauser SL, McVean G, Donnelly P, Compston A. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476(7359):214–9. doi:10.1038/nature10251.

81. Jarius S, Paul F, Ruprecht K, Wildemann B. Low vitamin B12 levels and gastric parietal cell antibodies in patients with aquaporin-4 antibody-positive neuromyelitis optica spectrum disorders. J Neurol. 2012;259(12):2743–5. doi:10.1007/s00415-012-6677-1.
82. Kao JY, Zhang M, Miller MJ, Mills JC, Wang B, Liu M, Eaton KA, Zou W, Berndt BE, Cole TS, Takeuchi T, Owyang SY, Luther J. *Helicobacter pylori* immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology. 2010;138(3):1046–54. doi:10.1053/j.gastro.2009.11.043.
83. Khamri W, Walker MM, Clark P, Atherton JC, Thursz MR, Bamford KB, Lechler RI, Lombardi G. *Helicobacter pylori* stimulates dendritic cells to induce interleukin-17 expression from CD4+ T lymphocytes. Infect Immun. 2010;78(2):845–53. doi:10.1128/IAI.00524-09.
84. Kidd M, Lastovica AJ, Atherton JC, Louw JA. Heterogeneity in the *Helicobacter pylori vacA* and *cagA* genes: association with gastroduodenal disease in South Africa? Gut. 1999; 45(4):499–502.
85. Kido M, Watanabe N, Aoki N, Iwamoto S, Nishiura H, Maruoka R, Ikeda A, Azuma T, Chiba T. Dual roles of CagA protein in *Helicobacter pylori*-induced chronic gastritis in mice. Biochem Biophys Res Commun. 2011;412(2):266–72. doi:10.1016/j.bbrc.2011.07.081.
86. Kim JM, Kim JS, Yoo DY, Ko SH, Kim N, Kim H, Kim YJ. Stimulation of dendritic cells with *Helicobacter pylori* vacuolating cytotoxin negatively regulates their maturation via the restoration of E2F1. Clin Exp Immunol. 2011;166(1):34–45. doi:10.1111/j.1365-2249.2011.04447.x.
87. Kodaman N, Pazos A, Schneider BG, Piazuelo MB, Mera R, Sobota RS, Sicinschi LA, Shaffer CL, Romero-Gallo J, de Sablet T, Harder RH, Bravo LE, Peek Jr RM, Wilson KT, Cover TL, Williams SM, Correa P. Human and *Helicobacter pylori* coevolution shapes the risk of gastric disease. Proc Natl Acad Sci U S A. 2014;111(4):1455–60. doi:10.1073/pnas.1318093111.
88. Krakowiak MS, Noto JM, Piazuelo MB, Hardbower DM, Romero-Gallo J, Delgado A, Chaturvedi R, Correa P, Wilson KT, Peek Jr RM. Matrix metalloproteinase 7 restrains *Helicobacter pylori*-induced gastric inflammation and premalignant lesions in the stomach by altering macrophage polarization. Oncogene. 2014. doi:10.1038/onc.2014.135.
89. Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W, Backert S. *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature. 2007;449(7164):862–6. doi:10.1038/nature06187.
90. Lebwohl B, Blaser MJ, Ludvigsson JF, Green PH, Rundle A, Sonnenberg A, Genta RM. Decreased risk of celiac disease in patients with *Helicobacter pylori* colonization. Am J Epidemiol. 2013;178(12):1721–30. doi:10.1093/aje/kwt234.
91. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4615–22. doi:10.1073/pnas.1000082107.
92. Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J Med Microbiol. 1988;26(2):93–9.
93. Li W, Minohara M, Piao H, Matsushita T, Masaki K, Matsuoka T, Isobe N, Su JJ, Ohyagi Y, Kira J. Association of anti-*Helicobacter pylori* neutrophil-activating protein antibody response with anti-aquaporin-4 autoimmunity in Japanese patients with multiple sclerosis and neuromyelitis optica. Mult Scler. 2009;15(12):1411–21. doi:10.1177/1352458509348961.
94. Li W, Minohara M, Su JJ, Matsuoka T, Osoegawa M, Ishizu T, Kira J. *Helicobacter pylori* infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. J Neuroimmunol. 2007;184(1–2):227–31.
95. Lindgren A, Yun CH, Sjoling A, Berggren C, Sun JB, Jonsson E, Holmgren J, Svennerholm AM, Lundin SB. Impaired IFN-gamma production after stimulation with bacterial components by natural killer cells from gastric cancer patients. Exp Cell Res. 2011;317(6):849–58. doi:10.1016/j.yexcr.2011.01.006.
96. Liston A, Kohler RE, Townley S, Haylock-Jacobs S, Comerford I, Caon AC, Webster J, Harrison JM, Swann J, Clark-Lewis I, Korner H, McColl SR. Inhibition of CCR6 function reduces the severity of experimental autoimmune encephalomyelitis via effects on the priming phase of the immune response. J Immunol. 2009;182(5):3121–30. doi:10.4049/jimmunol.0713169.

97. Liu Y, Carlsson R, Comabella M, Wang J, Kosicki M, Carrion B, Hasan M, Wu X, Montalban X, Dziegiel MH, Sellebjerg F, Sorensen PS, Helin K, Issazadeh-Navikas S. FoxA1 directs the lineage and immunosuppressive properties of a novel regulatory T cell population in EAE and MS. Nat Med. 2014;20(3):272–82. doi:10.1038/nm.3485.
98. Long Y, Gao C, Qiu W, Hu X, Shu Y, Peng F, Lu Z. *Helicobacter pylori* infection in Neuromyelitis Optica and Multiple Sclerosis. Neuroimmunomodulation. 2013;20(2):107–12. doi:10.1159/000345838.
99. Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta. 2011;1812(2):246–51. doi:10.1016/j.bbadis.2010.05.012.
100. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sorensen PS, Thompson AJ, Wolinsky JS, Balcer LJ, Banwell B, Barkhof F, Bebo Jr B, Calabresi PA, Clanet M, Comi G, Fox RJ, Freedman MS, Goodman AD, Inglese M, Kappos L, Kieseier BC, Lincoln JA, Lubetzki C, Miller AE, Montalban X, O'Connor PW, Petkau J, Pozzilli C, Rudick RA, Sormani MP, Stuve O, Waubant E, Polman CH. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83(3):278–86. doi:10.1212/WNL.0000000000000560.
101. Lundgren A, Stromberg E, Sjoling A, Lindholm C, Enarsson K, Edebo A, Johnsson E, Suri-Payer E, Larsson P, Rudin A, Svennerholm AM, Lundin BS. Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in *Helicobacter pylori*-infected patients. Infect Immun. 2005;73(1):523–31.
102. Luther J, Owyang SY, Takeuchi T, Cole TS, Zhang M, Liu M, Erb-Downward J, Rubenstein JH, Chen CC, Pierzchala AV, Paul JA, Kao JY. *Helicobacter pylori* DNA decreases pro-inflammatory cytokine production by dendritic cells and attenuates dextran sodium sulphate-induced colitis. Gut. 2011;60(11):1479–86. doi:10.1136/gut.2010.220087.
103. Macarthur M, Hold GL, El-Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol. 2004;286(4):G515–20.
104. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol. 2015;14(2):183–93. doi:10.1016/S1474-4422(14)70256-X.
105. Malaty HM. Epidemiology of *Helicobacter pylori* infection. In: Sutton P, Mitchell HM, editors. *Helicobacter pylori* in the 21st century. Oxfordshire: CABI; 2010. p. 1–12.
106. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. BMJ. 2000;320(7232):412–7.
107. Matsukawa Y, Asai Y, Kitamura N, Sawada S, Kurosaka H. Exacerbation of rheumatoid arthritis following *Helicobacter pylori* eradication: disruption of established oral tolerance against heat shock protein? Med Hypotheses. 2005;64(1):41–3.
108. Matsuzaki J, Suzuki H, Minegishi Y, Sugai E, Tsugawa H, Yasui M, Hibi T. Acid suppression by proton pump inhibitors enhances aquaporin-4 and KCNQ1 expression in gastric fundic parietal cells in mouse. Dig Dis Sci. 2010;55(12):3339–48. doi:10.1007/s10620-010-1167-8.
109. Mayo L, Trauger SA, Blain M, Nadeau M, Patel B, Alvarez JI, Mascanfroni ID, Yeste A, Kivisakk P, Kallas K, Ellezam B, Bakshi R, Prat A, Antel JP, Weiner HL, Quintana FJ. Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. Nat Med. 2014;20(10):1147–56. doi:10.1038/nm.3681.
110. Memon AA, Hussein NR, Miendje Deyi VY, Burette A, Atherton JC. Vacuolating cytotoxin genotypes are strong markers of gastric cancer and duodenal ulcer-associated *Helicobacter pylori* strains: a matched case-control study. J Clin Microbiol. 2014;52(8):2984–9. doi:10.1128/JCM.00551-14.
111. Miehlke S, Kirsch C, Agha-Amiri K, Gunther T, Lehn N, Malfertheiner P, Stolte M, Ehninger G, Bayerdorffer E. The *Helicobacter pylori vacA s1, m1* genotype and *cagA* is associated with gastric carcinoma in Germany. Int J Cancer. 2000;87(3):322–7.
112. Mizuno H, Sato S, Ohnishi Y, Takahashi T, Nakashima I, Fujihara K, Aoki M, Ohnuma A. A case of myelitis with anti-aquaporin 4 antibody concomitant with immune thrombocytopenic purpura. Rinsho Shinkeigaku. 2014;54(3):195–9.

113. Mohebi N, Mamarabadi M, Moghaddasi M. Relation of *Helicobacter pylori* infection and multiple sclerosis in Iranian patients. Neurol Int. 2013;5(2):31–3. doi:10.4081/ni.2013.e10.
114. Molinari M, Salio M, Galli C, Norais N, Rappuoli R, Lanzavecchia A, Montecucco C. Selective inhibition of Ii-dependent antigen presentation by *Helicobacter pylori* toxin VacA. J Exp Med. 1998;187(1):135–40.
115. Montgomery SM, Lambe M, Olsson T, Ekbom A. Parental age, family size, and risk of multiple sclerosis. Epidemiology. 2004;15(6):717–23.
116. Moran AP, Lindner B, Walsh EJ. Structural characterization of the lipid A component of *Helicobacter pylori* rough- and smooth-form lipopolysaccharides. J Bacteriol. 1997; 179(20):6453–63.
117. Mortimer K, Brown A, Feary J, Jagger C, Lewis S, Antoniak M, Pritchard D, Britton J. Dose-ranging study for trials of therapeutic infection with *Necator americanus* in humans. Am J Trop Med Hyg. 2006;75(5):914–20.
118. Muhl H, Bachmann M, Pfeilschifter J. Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity. Cell Microbiol. 2011;13(3):340–8. doi:10.1111/j.1462-5822.2010.01559.x.
119. Munari F, Fassan M, Capitani N, Codolo G, Vila-Caballer M, Pizzi M, Rugge M, Della Bella C, Troilo A, D'Elios S, Baldari CT, D'Elios MM, de Bernard M. Cytokine BAFF released by *Helicobacter pylori*-infected macrophages triggers the Th17 response in human chronic gastritis. J Immunol. 2014;193(11):5584–94. doi:10.4049/jimmunol.1302865.
120. Murphy AC, Lalor SJ, Lynch MA, Mills KH. Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis. Brain Behav Immun. 2010;24(4):641–51. doi:10.1016/j.bbi.2010.01.014.
121. Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ. *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol. 2001;158(2):647–54.
122. Nomura AM, Lee J, Stemmermann GN, Nomura RY, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* CagA seropositivity and gastric carcinoma risk in a Japanese American population. J Infect Dis. 2002;186(8):1138–44. doi:10.1086/343808.
123. Nomura AM, Perez-Perez GI, Lee J, Stemmermann G, Blaser MJ. Relation between *Helicobacter pylori cagA* status and risk of peptic ulcer disease. Am J Epidemiol. 2002;155(11):1054–9.
124. Nylander A, Hafler DA. Multiple sclerosis. J Clin Invest. 2012;122(4):1180–8. doi:10.1172/JCI58649.
125. O'Keeffe J, Gately CM, O'Donoghue Y, Zulquernain SA, Stevens FM, Moran AP. Natural killer cell receptor T-lymphocytes in normal and *Helicobacter pylori*-infected human gastric mucosa. Helicobacter. 2008;13(6):500–5.
126. Ochoa-Reparaz J, Mielcarz DW, Wang Y, Begum-Haque S, Dasgupta S, Kasper DL, Kasper LH. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. Mucosal Immunol. 2010;3(5):487–95. doi:10.1038/mi.2010.29.
127. Odenbreit S, Kavermann H, Puls J, Haas R. CagA tyrosine phosphorylation and interleukin-8 induction by *Helicobacter pylori* are independent from alpAB, HopZ and bab group outer membrane proteins. Int J Med Microbiol. 2002;292(3–4):257–66.
128. Oertli M, Noben M, Engler DB, Semper RP, Reuter S, Maxeiner J, Gerhard M, Taube C, Muller A. *Helicobacter pylori* gamma-glutamyl transpeptidase and vacuolating cytotoxin promote gastric persistence and immune tolerance. Proc Natl Acad Sci U S A. 2013;110(8):3047–52. doi:10.1073/pnas.1211248110.
129. Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, Maxeiner J, Hansson M, Taube C, Quiding-Jarbrink M, Muller A. DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-specific immune tolerance, and asthma protection. J Clin Invest. 2012;122(3):1082–96. 61029 [pii]. doi:10.1172/JCI61029.
130. Parkin DM. 11. Cancers attributable to infection in the UK in 2010. Br J Cancer. 2011;105 Suppl 2:S49–56. doi:10.1038/bjc.2011.484.

131. Paul F, Jarius S, Aktas O, Bluthner M, Bauer O, Appelhans H, Franciotta D, Bergamaschi R, Littleton E, Palace J, Seelig HP, Hohlfeld R, Vincent A, Zipp F. Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. PLoS Med. 2007;4(4), e133. doi:10.1371/journal.pmed.0040133.
132. Paziak-Domanska B, Chmiela M, Jarosinska A, Rudnicka W. Potential role of CagA in the inhibition of T cell reactivity in *Helicobacter pylori* infections. Cell Immunol. 2000;202(2):136–9.
133. Peek Jr RM, Fiske C, Wilson KT. Role of innate immunity in *Helicobacter pylori*-induced gastric malignancy. Physiol Rev. 2010;90(3):831–58. doi:10.1152/physrev.00039.2009.
134. Peek Jr RM, Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, Blaser MJ. Heightened inflammatory response and cytokine expression in vivo to *cagA*+ *Helicobacter pylori* strains. Lab Invest. 1995;73(6):760–70.
135. Peleteiro B, Bastos A, Ferro A, Lunet N. Prevalence of *Helicobacter pylori* infection worldwide: a systematic review of studies with national coverage. Dig Dis Sci. 2014;59(8):1698–709. doi:10.1007/s10620-014-3063-0.
136. Pezeshki MZ, Zarrintan S, Zarrintan MH. *Helicobacter pylori* nanoparticles as a potential treatment of conventional multiple sclerosis. Med Hypotheses. 2008;70(6):1223. doi:10.1016/j.mehy.2007.12.008.
137. Pinto-Santini D, Salama NR. The biology of *Helicobacter pylori* infection, a major risk factor for gastric adenocarcinoma. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1853–8. doi:10.1158/1055-9965.EPI-04-0784.
138. Podda G, Nyirenda M, Crooks J, Gran B. Innate immune responses in the CNS: role of toll-like receptors, mechanisms, and therapeutic opportunities in multiple sclerosis. J Neuroimmune Pharmacol. 2013;8(4):791–806. doi:10.1007/s11481-013-9483-3.
139. Popova A, Kzhyshkowska J, Nurgazieva D, Goerdt S, Gratchev A. Pro- and anti-inflammatory control of M-CSF-mediated macrophage differentiation. Immunobiology. 2011;216(1–2):164–72. doi:10.1016/j.imbio.2010.06.003.
140. Quiding-Jarbrink M, Lundin BS, Lonroth H, Svennerholm AM. CD4+ and CD8+ T cell responses in *Helicobacter pylori*-infected individuals. Clin Exp Immunol. 2001;123(1):81–7.
141. Quiding-Jarbrink M, Raghavan S, Sundquist M. Enhanced M1 macrophage polarization in human *Helicobacter pylori*-associated atrophic gastritis and in vaccinated mice. PLoS One. 2010;5(11), e15018. doi:10.1371/journal.pone.0015018.
142. Quintana FJ, Patel B, Yeste A, Nyirenda M, Kenison J, Rahbari R, Fetco D, Hussain M, O'Mahony J, Magalhaes S, McGowan M, Johnson T, Rajasekharan S, Narayanan S, Arnold DL, Weiner HL, Banwell B, Bar-Or A, Canadian Pediatric Demyelinating Disease N. Epitope spreading as an early pathogenic event in pediatric multiple sclerosis. Neurology. 2014;83(24):2219–26. doi:10.1212/WNL.0000000000001066.
143. Rad R, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C. CD25+/Foxp3+ T cells regulate gastric inflammation and *Helicobacter pylori* colonization in vivo. Gastroenterology. 2006;131(2):525–37.
144. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology. 2007;133(3):926–36.
145. Rimbara E, Mori S, Kim H, Shibayama K. Role of gamma-glutamyltranspeptidase in the pathogenesis of *Helicobacter pylori* infection. Microbiol Immunol. 2013;57(10):665–73. doi:10.1111/1348-0421.12089.
146. Robinson K, Argent RH, Atherton JC. The inflammatory and immune response to *Helicobacter pylori* infection. Best Pract Res Clin Gastroenterol. 2007;21(2):237–59.
147. Robinson K, Atherton J. *Helicobacter pylori*. In: Fratamico PM, Smith JL, Brogden KA, editors. Sequelae and long-term consequences of infectious diseases. Washington, DC: ASM Press; 2009. p. 107–33.
148. Robinson K, Kenefeck R, Pidgeon EL, Shakib S, Patel S, Polson RJ, Zaitoun AM, Atherton JC. *Helicobacter pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. Gut. 2008;57(10):1375–85.

149. Rook GA. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42(1):5–15. doi:10.1007/s12016-011-8285-8.
150. Rook GA. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. Proc Natl Acad Sci U S A. 2013;110(46):18360–7. doi:10.1073/pnas.1313731110.
151. Rubenstein JH, Inadomi JM, Scheiman J, Schoenfeld P, Appelman H, Zhang M, Metko V, Kao JY. Association between *Helicobacter pylori* and Barrett's esophagus, erosive esophagitis, and gastroesophageal reflux symptoms. Clin Gastroenterol Hepatol. 2014;12(2):239–45. doi:10.1016/j.cgh.2013.08.029.
152. Rudi J, Kolb C, Maiwald M, Kuck D, Sieg A, Galle PR, Stremmel W. Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J Clin Microbiol. 1998;36(4):944–8.
153. Rudnicka K, Matusiak A, Miszczyk E, Rudnicka W, Tenderenda M, Chmiela M. Immunophenotype of peripheral blood natural killer cells and IL-10 serum levels in relation to *Helicobacter pylori* status. APMIS. 2013;121(9):806–13. doi:10.1111/apm.12120.
154. Rudnicka K, Miszczyk E, Matusiak A, Walencka M, Moran AP, Rudnicka W, Chmiela M. *Helicobacter pylori*-driven modulation of NK cell expansion, intracellular cytokine expression and cytotoxic activity. Innate Immun. 2015;21(2):127–39. doi:10.1177/1753425913518225.
155. Sagar D, Lamontagne A, Foss CA, Khan ZK, Pomper MG, Jain P. Dendritic cell CNS recruitment correlates with disease severity in EAE via CCL2 chemotaxis at the blood-brain barrier through paracellular transmigration and ERK activation. J Neuroinflammation. 2012;9:245. doi:10.1186/1742-2094-9-245.
156. Saito A, Yokohama A, Osaki Y, Ogawa Y, Nakahashi H, Toyama K, Mitsui T, Hashimoto Y, Koiso H, Uchiumi H, Saitoh T, Handa H, Sawamura M, Karasawa M, Murakami H, Tsukamoto N, Nojima Y. Circulating plasmacytoid dendritic cells in patients with primary and *Helicobacter pylori*-associated immune thrombocytopenia. Eur J Haematol. 2012;88(4):340–9. doi:10.1111/j.1600-0609.2011.01745.x.
157. Salama NR, Hartung ML, Muller A. Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Nat Rev Microbiol. 2013;11(6):385–99. doi:10.1038/nrmicro3016.
158. Sawalha AH, Schmid WR, Binder SR, Bacino DK, Harley JB. Association between systemic lupus erythematosus and *Helicobacter pylori* seronegativity. J Rheumatol. 2004;31(8):1546–50.
159. Saxena A, Martin-Blondel G, Mars LT, Liblau RS. Role of CD8 T cell subsets in the pathogenesis of multiple sclerosis. FEBS Lett. 2011;585(23):3758–63. doi:10.1016/j.febslet.2011.08.047.
160. Schmees C, Gerhard M, Treptau T, Voland P, Schwendy S, Rad R, Prinz C. VacA-associated inhibition of T-cell function: reviewed and reconsidered. Helicobacter. 2006;11(3):144–6. doi:10.1111/j.1523-5378.2006.00393.x.
161. Schmees C, Prinz C, Treptau T, Rad R, Hengst L, Voland P, Bauer S, Brenner L, Schmid RM, Gerhard M. Inhibition of T-cell proliferation by *Helicobacter pylori* gamma-glutamyl transpeptidase. Gastroenterology. 2007;132(5):1820–33.
162. Schwartz JT, Allen LA. Role of urease in megasome formation and *Helicobacter pylori* survival in macrophages. J Leukoc Biol. 2006;79(6):1214–25.
163. Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, Virta M, Hurme M, Uibo R, Knip M, Hyoty H. Allergic sensitization and microbial load – a comparison between Finland and Russian Karelia. Clin Exp Immunol. 2007;148(1):47–52.
164. Sellebjerg F, Krakauer M, Khademi M, Olsson T, Sorensen PS. FOXP3, CBLB and ITCH gene expression and cytotoxic T lymphocyte antigen 4 expression on CD4(+) CD25(high) T cells in multiple sclerosis. Clin Exp Immunol. 2012;170(2):149–55. doi:10.1111/j.1365-2249.2012.04654.x.
165. Serelli-Lee V, Ling KL, Ho C, Yeong LH, Lim GK, Ho B, Wong SB. Persistent *Helicobacter pylori* specific Th17 responses in patients with past H. pylori infection are associated with elevated gastric mucosal IL-1beta. PLoS One. 2012;7(6):e39199. doi:10.1371/journal.pone.0039199.

166. Serrano C, Wright SW, Bimczok D, Shaffer CL, Cover TL, Venegas A, Salazar MG, Smythies LE, Harris PR, Smith PD. Downregulated Th17 responses are associated with reduced gastritis in *Helicobacter pylori*-infected children. Mucosal Immunol. 2013;6(5):950–9. doi:10.1038/mi.2012.133.
167. Sewald X, Gebert-Vogl B, Prassl S, Barwig I, Weiss E, Fabbri M, Osicka R, Schiemann M, Busch DH, Semmrich M, Holzmann B, Sebo P, Haas R. Integrin subunit CD18 Is the T-lymphocyte receptor for the *Helicobacter pylori* vacuolating cytotoxin. Cell Host Microbe. 2008;3(1):20–9. doi:10.1016/j.chom.2007.11.003.
168. Shi Y, Liu XF, Zhuang Y, Zhang JY, Liu T, Yin Z, Wu C, Mao XH, Jia KR, Wang FJ, Guo H, Flavell RA, Zhao Z, Liu KY, Xiao B, Guo Y, Zhang WJ, Zhou WY, Guo G, Zou QM. *Helicobacter pylori*-induced Th17 responses modulate Th1 cell responses, benefit bacterial growth, and contribute to pathology in mice. J Immunol. 2010;184(9):5121–9. doi:10.4049/jimmunol.0901115.
169. Smith SM. Role of toll-like receptors in *Helicobacter pylori* infection and immunity. World J Gastrointest Pathophysiol. 2014;5(3):133–46. doi:10.4291/wjgp.v5.i3.133.
170. Smith SM, Moran AP, Duggan SP, Ahmed SE, Mohamed AS, Windle HJ, O'Neill LA, Kelleher DP. Tribbles 3: a novel regulator of TLR2-mediated signaling in response to *Helicobacter pylori* lipopolysaccharide. J Immunol. 2011;186(4):2462–71. doi:10.4049/jimmunol.1000864.
171. Smyk DS, Koutsoumpas AL, Mytilinaiou MG, Rigopoulou EI, Sakkas LI, Bogdanos DP. *Helicobacter pylori* and autoimmune disease: cause or bystander. World J Gastroenterol. 2014;20(3):613–29. doi:10.3748/wjg.v20.i3.613.
172. Sommer F, Faller G, Konturek P, Kirchner T, Hahn EG, Zeus J, Rollinghoff M, Lohoff M. Antrum- and corpus mucosa-infiltrating CD4(+) lymphocytes in *Helicobacter pylori* gastritis display a Th1 phenotype. Infect Immun. 1998;66(11):5543–6.
173. Sormani MP, Arnold DL, De Stefano N. Treatment effect on brain atrophy correlates with treatment effect on disability in multiple sclerosis. Ann Neurol. 2014;75(1):43–9. doi:10.1002/ana.24018.
174. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299(6710):1259–60.
175. Sundquist M, Quiding-Jarbrink M. *Helicobacter pylori* and its effect on innate and adaptive immunity: new insights and vaccination strategies. Expert Rev Gastroenterol Hepatol. 2010;4(6):733–44. doi:10.1586/egh.10.71.
176. Sundrud MS, Torres VJ, Unutmaz D, Cover TL. Inhibition of primary human T cell proliferation by *Helicobacter pylori* vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci U S A. 2004;101(20):7727–32.
177. t Hart BA, Gran B, Weissert R. EAE: imperfect but useful models of multiple sclerosis. Trends Mol Med. 2011;17(3):119–25. doi:10.1016/j.molmed.2010.11.006.
178. Tan MP, Pedersen J, Zhan Y, Lew AM, Pearse MJ, Wijburg OL, Strugnell RA. CD8+ T cells are associated with severe gastritis in *Helicobacter pylori*-infected mice in the absence of CD4+ T cells. Infect Immun. 2008;76(3):1289–97.
179. Tanasescu R, Constantinescu CS. Helminth therapy for MS. Curr Top Behav Neurosci. 2014. doi:10.1007/7854_2014_361.
180. Tegtmeyer N, Wessler S, Backert S. Role of the *cag*-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J. 2011;278(8):1190–202. doi:10.1111/j.1742-4658.2011.08035.x.
181. Teymournejad O, Mobarez AM, Hassan ZM, Moazzeni SM, Ahmadabad HN. In vitro suppression of dendritic cells by *Helicobacter pylori* OipA. Helicobacter. 2014;19(2):136–43. doi:10.1111/hel.12107.
182. Torres VJ, VanCompernolle SE, Sundrud MS, Unutmaz D, Cover TL. *Helicobacter pylori* vacuolating cytotoxin inhibits activation-induced proliferation of human T and B lymphocyte subsets. J Immunol. 2007;179(8):5433–40.
183. Trujillo C, Erb KJ. Inhibition of allergic disorders by infection with bacteria or the exposure to bacterial products. Int J Med Microbiol. 2003;293(2–3):123–31.

184. Valsasina P, Rocca MA, Horsfield MA, Absinta M, Messina R, Caputo D, Comi G, Filippi M. Regional cervical cord atrophy and disability in multiple sclerosis: a voxel-based analysis. Radiology. 2013;266(3):853–61. doi:10.1148/radiol.12120813.
185. Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Memet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. Nat Immunol. 2004;5(11):1166–74.
186. Vyse AJ, Gay NJ, Hesketh LM, Andrews NJ, Marshall B, Thomas HI, Morgan-Capner P, Miller E. The burden of *Helicobacter pylori* infection in England and Wales. Epidemiol Infect. 2002;128(3):411–7.
187. Wang SK, Zhu HF, He BS, Zhang ZY, Chen ZT, Wang ZZ, Wu GL. CagA+ *H. pylori* infection is associated with polarization of T helper cell immune responses in gastric carcinogenesis. World J Gastroenterol. 2007;13(21):2923–31.
188. Wei L, Wang J, Liu Y. Prior to Foxp3(+) regulatory T-cell induction, interleukin-10-producing B cells expand after *Helicobacter pylori* infection. Pathog Dis. 2014;72(1):45–54. doi:10.1111/2049-632X.12182.
189. Wender M. Prevalence of *Helicobacter pylori* infection among patients with multiple sclerosis. Neurol Neurochir Pol. 2003;37(1):45–8.
190. Wilke CM, Bishop K, Fox D, Zou W. Deciphering the role of Th17 cells in human disease. Trends Immunol. 2011;32(12):603–11. doi:10.1016/j.it.2011.08.003.
191. Wilson KT, Crabtree JE. Immunology of *Helicobacter pylori*: insights into the failure of the immune response and perspectives on vaccine studies. Gastroenterology. 2007;133(1):288–308.
192. Wu YY, Tsai HF, Lin WC, Hsu PI, Shun CT, Wu MS, Hsu PN. Upregulation of CCL20 and recruitment of CCR6+ gastric infiltrating lymphocytes in *Helicobacter pylori* gastritis. Infect Immun. 2007;75(9):4357–63. IAI.01660-06 [pii]. doi:10.1128/IAI.01660-06.
193. Wustner S, Mejias-Luque R, Koch MF, Rath E, Vieth M, Sieber SA, Haller D, Gerhard M. *Helicobacter pylori* gamma-glutamyltranspeptidase impairs T-lymphocyte function by compromising metabolic adaption through inhibition of cMyc and IRF4 expression. Cell Microbiol. 2015;17(1):51–61. doi:10.1111/cmi.12335.
194. Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY. Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology. 2002;123(2):414–24.
195. Yamaoka Y, Kita M, Kodama T, Sawai N, Tanahashi T, Kashima K, Imanishi J. Chemokines in the gastric mucosa in *Helicobacter pylori* infection. Gut. 1998;42(5):609–17.
196. Yamaoka Y, Kwon DH, Graham DY. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. Proc Natl Acad Sci U S A. 2000;97(13):7533–8.
197. Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, El-Zimaity HM, Reddy R, Arnqvist A, Graham DY. *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. Gut. 2006;55(6):775–81.
198. Yin YN, Wang CL, Liu XW, Cui Y, Xie N, Yu QF, Li FJ, Lu FG. Gastric and duodenum microflora analysis after long-term *Helicobacter pylori* infection in Mongolian Gerbils. Helicobacter. 2011;16(5):389–97. doi:10.1111/j.1523-5378.2011.00862.x.
199. Yoshimura S, Isobe N, Matsushita T, Masaki K, Sato S, Kawano Y, Ochi H, Kira J. Genetic and infectious profiles influence cerebrospinal fluid IgG abnormality in Japanese multiple sclerosis patients. PLoS One. 2014;9(4), e95367. doi:10.1371/journal.pone.0095367.
200. Yoshimura S, Isobe N, Matsushita T, Yonekawa T, Masaki K, Sato S, Kawano Y, Kira J, South Japan Multiple Sclerosis Genetics C. Distinct genetic and infectious profiles in Japanese neuromyelitis optica patients according to anti-aquaporin 4 antibody status. J Neurol Neurosurg Psychiatry. 2013;84(1):29–34. doi:10.1136/jnnp-2012-302925.
201. Yoshimura S, Isobe N, Yonekawa T, Matsushita T, Masaki K, Sato S, Kawano Y, Yamamoto K, Kira J, South Japan Multiple Sclerosis Genetics C. Genetic and infectious profiles of Japanese multiple sclerosis patients. PLoS One. 2012;7(11), e48592. doi:10.1371/journal.pone.0048592.

Chapter 7
Stress and the Gastrointestinal System

Bruno Bonaz

Abstract Stress was characterized by Hans Selye, in 1936, as the "stereotyped biological response to any demand." Corticotrophin-releasing factor (CRF) and its related peptides urocortins (Ucns 1,2,3) are the mediators of stress. They are present both in the central nervous system and in the gastrointestinal (GI) tract where they exert their biological actions on target cells through activation of two receptors, CRF1 and CRF2. Stress is able to modulate numerous functions of the GI tract such as motility, secretion, permeability, sensitivity, and microbiota. Classically, stress delays gastric emptying while stimulating colonic transit and secretion, increases intestinal permeability and visceral sensitivity, and modifies intestinal microbiota. Through these various effects at the level of the GI tract, stress is involved in the pathophysiology of irritable bowel syndrome (IBS), a functional digestive disorder, as well as inflammatory bowel disease (IBD), Crohn's disease, and ulcerative colitis. Targeting these CRF1/CRF2 signaling pathways by selective antagonists/agonists should be of clinical interest in the domain of IBS and IBD.

Keywords Autonomic nervous system • Corticotrophin-releasing factor • Gastrointestinal • Hypothalamus • Inflammatory bowel disease • Irritable bowel syndrome • Stress • Sympathetic nervous system • Urocortins • Vagus nerve

Stress-Definition

In 1936, Hans Selye defined the concept of stress as the "stereotyped biological response to any demand" [168] and elaborated the concept of the general adaptation syndrome. Later, the hypothalamic factor named corticotrophin-releasing factor (CRF) which stimulates ACTH release by the rat pituitary was discovered [77], positioning the hypothalamic–pituitary adrenal (HPA) axis as a key element in this concept. In 1981, Vale and his group [196] identified the 41-aa peptide CRF characterized

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from ovine hypothalami and later the cloning of CRF receptors and the development of specific CRF receptor antagonists [157]. In 2003, McEwen and Wingfield [121] defined the concept of allostasis as a process through which organisms actively adjust to both predictable and unpredictable events with allostatic overload being the cause of serious pathophysiology.

History of Stress Influence on Gut Functions and Diseases

In 1833, William Beaumont [15], a US Army surgeon, was the first to describe the influence of fear and anger on gastric acid secretion of his patient Alexis St. Martin, a Canadian trapper with a permanent gastric fistula caused by a musket shot. In 1902, Walter Cannon [42] observed gastrointestinal (GI) motility disturbances in the cat faced with an aggressive dog. Numerous other stress models have confirmed the effect of stress on GI motility in rodents [185]. Nowadays, the effect of psychological, physical, and immunological stressors on GI secretion, motility, epithelial permeability, visceral sensitivity, microbiota, and inflammation is clearly demonstrated, and stress has a major influence in irritable bowel syndrome (IBS) and most likely plays a role in inflammatory bowel disease (IBD). The circuitries and biochemical coding, particularly CRF and its receptors, involved in the stress response have been characterized through *(i)* the use of the immediate early gene *c-fos*, a marker of neuronal activation, and its relation to the autonomic regulation of gut functions [33] and *(ii)* its combination with the identification of neuromediators activated by stress models and *(iii)* the use of CRF-overexpressing as well as knockout animals.

The present review will describe first the state of knowledge on the distribution of the CRFergic system including CRF and its related peptides urocortins (Ucns), and their CRF receptors, in the brain and in the GI tract and the effects and mechanisms of stress on gastrointestinal motility, secretion, permeability, visceral sensitivity, and microbiota. Lastly, the implication of stress in the pathophysiology of IBS and IBD [Crohn's disease (CD) and ulcerative colitis (UC)] will be highlighted.

Mechanisms of Stress

Stress Peptides and Their Receptors

CRF and CRF-Related Peptides (Fig. 7.1)

CRF is a member of a family of mammalian CRF-related peptides including Ucn 1, Ucn 2 (known as stresscopin-related peptide), and Ucn 3 (known as stresscopin) [12] with 40, 38, and 38 aa in length, respectively. During stress, CRF is released from

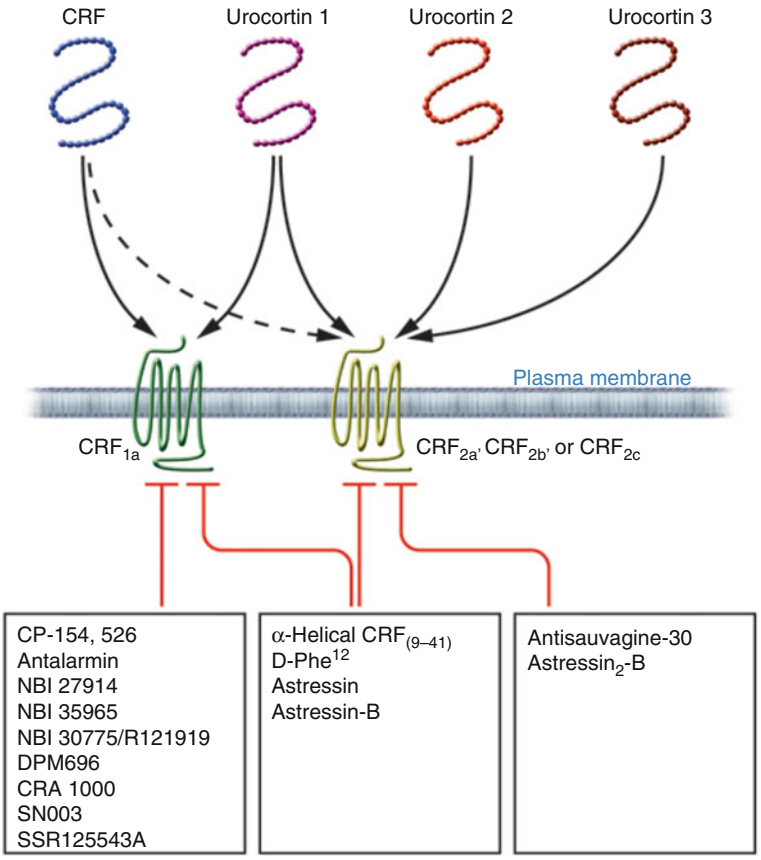

Fig. 7.1 The CRF peptide family and its receptors and receptor antagonists (With permission from Taché and Bonaz [185]). *CRF* corticotrophin-releasing factor

the hypothalamus and is vehiculated through a portal venous network to the anterior pituitary where it triggers the release of ACTH which stimulates the release of cortisol, the stress steroid, by the adrenal glands, i.e., the HPA axis (Fig. 7.2). CRF also acts directly in the central nervous system (CNS) as a neurotransmitter and neuromodulator through the projections of CRF-containing neurons to areas of the central autonomic network that controls the central response to stress. The intracerebral injection of CRF mimics behavioral (anxiety/depression, alterations of feeding), autonomic (sympathetic and sacral parasympathetic activation, vagal inhibition), immune, metabolic, and visceral responses induced by various systemic or cognitive stressors [12, 188]. The hypothalamic peptide arginine–vasopressin (AVP) acts in synergy with CRF to stimulate ACTH secretion in acute stress conditions, while a habituation of the hypothalamic CRF is observed in chronic stress conditions where AVP takes over from CRF [31].

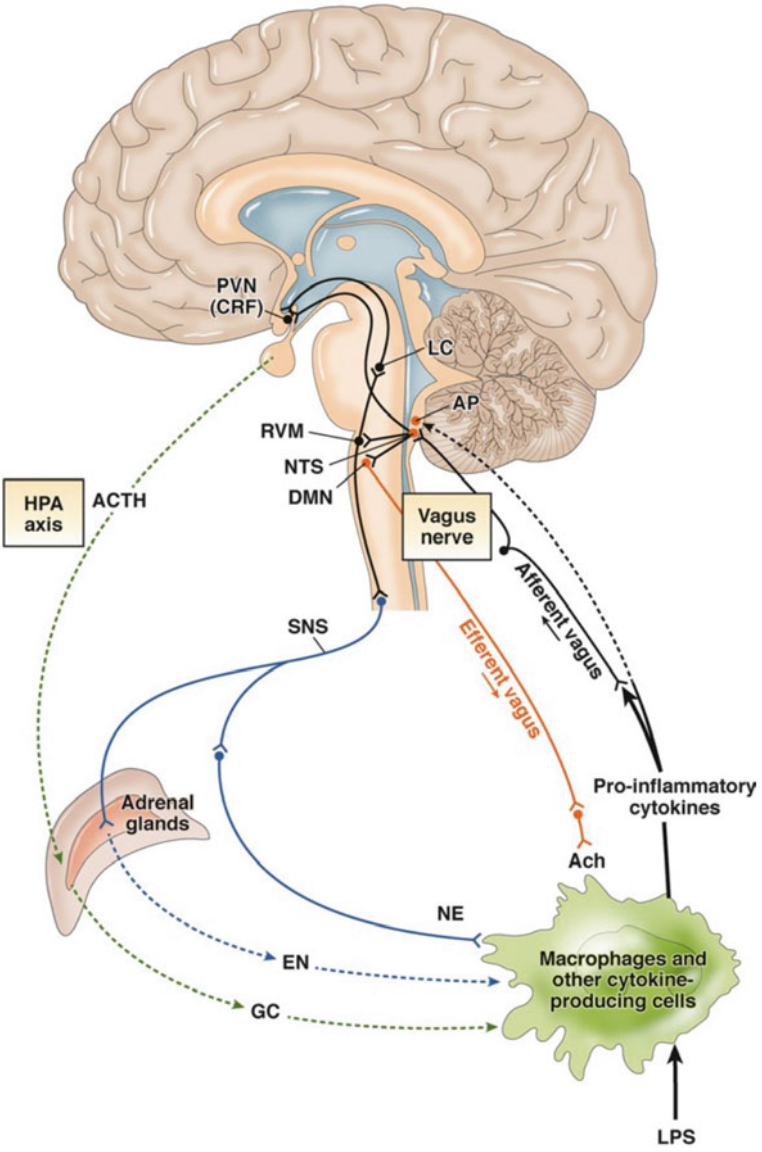

Fig. 7.2 The hypothalamic–pituitary–adrenal axis and the autonomic nervous system (With permission from Bonaz and Bernstein [27])). *ACTH* adrenocorticotropic hormone, *AP* area postrema, *CRF* corticotrophin-releasing factor, *DMN* dorsal motor nucleus of the vagus nerve, *EN* epinephrine, *GC* glucocorticoids, *HPA* hypothalamic–pituitary adrenal axis, *LC* locus coeruleus, *LPS* lipopolysaccharides, *NE* norepinephrine, *PVN* paraventricular nucleus of the hypothalamus, *RVM* rostral ventrolateral medulla

CRF Receptors

CRF and Ucns exert their biological actions on target cells through activation of two receptors, CRF1 (415 aa) and CRF2 (411 aa), that belong to the B1 class subfamily of 7-transmembrane domain G protein-coupled receptors and are encoded by two distinct genes [83].

CRF1a, the main functional variant of CRF1, is greatly expressed in the brain and some peripheral tissues in mammals [83]. Alternative splicing of the primary transcript encoding CRF1 can lead to other variants ($n=8$), named CRF1b–i, all of which display impaired signaling [83]. The functional significance of these other transcripts is still poorly characterized. Selective peptide CRF1 agonists (cortagine and stressin1-A) have been developed [155]. Three functional *CRF2* variants (2a,b,c) have been identified in humans [83], whereas only 2a and 2b are expressed in other mammals.

CRF1 and CRF2 have distinct affinities for the CRF family of peptides [12, 83]. CRF has a higher (10- to 40-fold) affinity for CRF1 than for CRF2. Ucn 1 binds CRF2 with a greater (100-fold) affinity than CRF and CRF1 with greater (sixfold) affinity than CRF. Ucns 2 and 3 exhibit high selectivity only for CRF2, with a slightly higher affinity for CRF2b *versus* CRF2a. CRF2a, CRF2b, and CRF2c are almost identical with high affinity for Ucn 1, Ucn 2, and Ucn 3 and lower affinity for rat/human CRF. In non-primate mammals, CRF2a is expressed only by neurons and CRF2b mainly in the periphery and by nonneuronal cells of the brain, whereas CRF2c is found only in the amygdala of the human brain. The binding of CRF family peptides to their receptors has been characterized [83]. The long N-terminal extracellular domain of CRF receptors primarily interacts with the C-terminal residues of CRF, and the N-terminal residues of CRF interact with the transmembrane region of the receptor, resulting in conformational changes that enable G protein activation. Stimulation of CRF1a, CRF2a, and CRF2b activates adenylyl cyclase/cAMP–protein kinase A signaling pathways through coupling and activation of Gαs proteins. CRF receptors modulate various kinases, including phosphokinases A, B, and C, and can phosphorylate and activate mitogen-activated protein kinase (MAPK), particularly the ERK1/2 and p38/MAPK pathways. CRF receptors also stimulate transient calcium mobilization in certain cell types by phospholipase C activation *in vitro* and PKC activation *in vivo*.

CRF receptor antagonists have been developed to characterize the functions of CRF receptors in the behavioral (anxiety, decreased feeding, and drug seeking), cognitive (arousal and anxiety), neuroendocrine (ACTH release), autonomic (activation of the sympathetic nervous system), immunological, and visceral (hypertension and alterations in gut motor function) responses to stress [50]. α-helical CRF9–41 was the first CRF receptor antagonist [157] followed by subsequent CRF antagonists such as d-Phe12CRF12–41 and astressin. Astressin-B was developed later, as the most efficacious and still effective 24 h after a single peripheral injection [158]. Peripheral administration of these peptide antagonists has a poor penetrance into the brain and could thus be used to selectively block physiological processes of the GI tract. Selective CRF2 peptide antagonists, binding equally to CRF2a, b, and c with little or no affinity for CRF1 receptors, have been developed

[156] such as antisauvagine-30 and the long-acting analog astressin2-B. Non-peptide selective CRF1 antagonists that cross the blood–brain barrier depending on their lipophilicity have been developed, e.g., CP-154,526, antalarmin, NBI 30775, and NBI 35965 [50]. The activation of the brain CRF/CRF1 signaling pathway plays a major role in the coordination of many physiological responses to adaptive stress through the activation of the HPA axis, autonomic nervous system (ANS), and changes in cardiovascular, GI, and immune functions in rodents and primates [80, 115]. The abnormal increase of central CRF1 signaling could contribute to the pathogenesis of anxiety and depression as well as to IBS [11, 80, 161]. CRF2 receptors in the brain dampen and/or facilitate the proper recovery of the CRF1-initiated behavioral, endocrine, and visceral responses to stress [154] and have a primary role in the anorexic response to exogenous administration of CRF and Ucns [177]. CRF-binding protein (332-aa) isolated in different species functions as an endogenous antagonist by sequestrating CRF ligands, thus modulating the access of CRF and related peptides to CRF receptors [16].

Expression of the CRFergic System in the Central Nervous System and the Gastrointestinal Tract

In the brain, these peptides are expressed in distinct regions. CRF is essentially expressed by the neurons of the parvocellular part of the paraventricular nucleus of the hypothalamus (PVH) involved in the activation of the HPA axis, in the prefrontal and cingulate cortices which mediate behavioral and cognitive components of stress, as well as in the central nucleus of the amygdala (CeA) [182], a limbic structure involved in the processing of emotion [29]. CRF is also present in the Barrington's nucleus, which is located adjacent to the locus coeruleus (LC) and regulates the sacral parasympathetic nucleus and thus the autonomic control of the rectum, left colon, and bladder [199]. CRF is also found in the hippocampus, bed nucleus of the stria terminalis, nucleus accumbens, several thalamic areas, substantia nigra, raphe, periaqueductal gray, cerebellum, and spinal cord.

Ucn 1 neurons are mainly localized in the Edinger–Westphal nucleus (EWN) and lateral superior olive of the pons in rodents, humans, and nonhuman primates and at a lesser extent in the olfactory bulb, supraoptic nucleus (SON), ventromedial and magnocellular regions of the PVH, lateral hypothalamic area, several cranial nerve motor nuclei, and in neurons of the ventral horn of the spinal cord in experimental animals and humans [25, 97]. In the rat, the highest density of Ucn 1 immunoreactive fibers is found in the lateral septum, with more moderate levels in the SON, PVH, periaqueductal gray, EWN, dorsal raphe, nucleus of the solitary tract (NTS), parabrachial nucleus, substantia nigra, and interpeduncular nucleus, as well as throughout the length of the spinal cord. The only Ucn 1 projections are those from the EWN to the spinal cord and lateral septum. There is little neuroanatomical overlap between the brain distribution of CRF and Ucn 1.

Ucn 2 mRNA is expressed in the PVH, SON, and arcuate nucleus of the hypothalamus and in the LC as well as in several cranial nerve motor nuclei and the

ventral horn of the spinal cord [113, 152]. The neuroanatomical distribution of Ucn 2-containing fibers has not been characterized.

The distribution of Ucn 3 in the brain differs from that of CRF and Ucn 1 and Ucn 2. Ucn 3 is essentially located in the median preoptic nucleus, perifornical area between the fornix and the PVH, dorsal division of the medial amygdaloid nucleus, and superior paraolivary nucleus [107, 113]. Major Ucn 3 terminal fields are found in the forebrain, including the amygdala, the lateral septum, and the ventromedial hypothalamus.

CRF and Ucn 1 and Ucn 2 are localized in several stress-related nuclei involved in the modulation of GI functions and the integration of afferent signals from visceral origin, such as the PVH, SON, Barrington nucleus, and LC.

CRF ligands and receptors are also expressed in the *GI tract* in animals and humans where they can act directly on the gut in a paracrine or autocrine manner, thus supporting a role for peripheral CRF and/or Ucns in the modulation of GI functions [38, 47, 48, 143, 144, 178].

CRF is present in guinea pig cecal smooth muscle cells and in epithelial and submucosal immune cells of the GI tract as well as in epithelial cells of the colonic mucosa including endocrine (enterochromaffin) cells, in the myenteric and submucosal plexuses along the GI tract. Numerous CRF-containing nerve terminals reached the circular smooth muscle layer and submucosal arterioles. *Ucn 1* has been described in the rat duodenum and in human gastric tissue, in parietal cells and others, as well as in the human colonic mucosa and in the submucosal and myenteric plexuses along the small intestine and colon of the rat. *Ucn 2* is mainly distributed in the stomach, while low levels are observed in the small intestine and colon. In contrast, Ucn 3 is highly expressed in the gut, mainly in the stomach, small intestine, and colon, but not in the ileocecal region, cecum, and esophagus.

CRF receptors: CRF1 is present in the rat colon in goblet and stem cells of the crypts as well as on surface epithelial cells, lamina propria, and preferentially in the myenteric nervous plexus. CRF1 and CRF2 are expressed in the lamina propria of the human colon [133], particularly at the level of the colonic myenteric plexus [125]. CRF2a mRNA is expressed in human colonic epithelial HT-29 cells, with little expression of the b and c splice variants [95]. CRF2 receptors are highly expressed in the rat upper gut, including the esophagus and stomach.

Neuroanatomy of the Brain–Gut Axis (Figs. 7.2 and 7.3)

The CNS and the gut communicate bidirectionally through the *ANS* (i.e., the brain–gut axis) and the circumventricular organs. The gut contains a "little brain," i.e., the enteric nervous system (ENS), which contains as many as neurons as in the spinal cord (400–600 million) and can function independently of the CNS for the programming of motility and secretion [66]. Some neuropeptides and receptors are present in both the ENS and the CNS. The function of the GI tract is modulated by both the ENS and the ANS which is composed of the sympathetic (i.e., the splanchnic nerves) and parasympathetic nervous system, i.e., the vagus nerves (VN), the largest visceral sensory nerve in the body, and the sacral parasympathetic nucleus represented by the

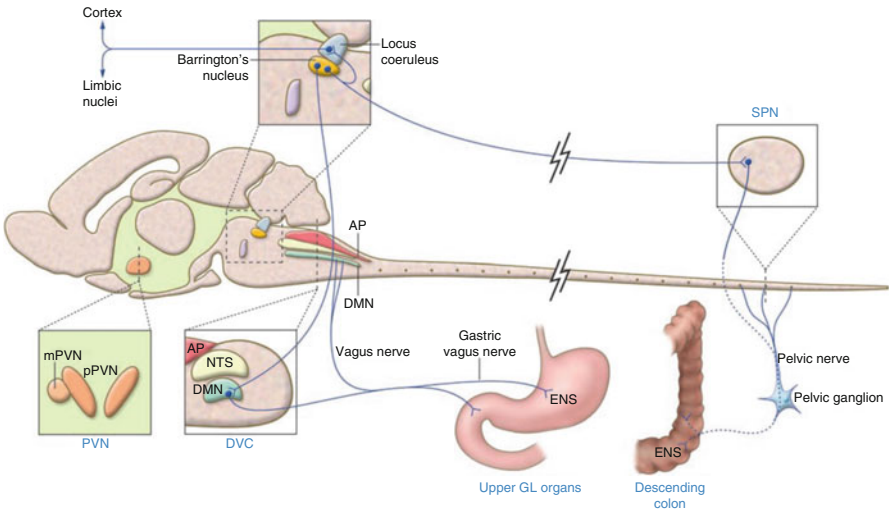

Fig. 7.3 Regions where the activation of CRF receptors influences gastric and colonic motor functions through neural pathways innervating the gut (With permission from Taché and Bonaz [185]). *AP* area postrema, *DMN* dorsal motor nucleus of the vagus nerve, *DVC* dorsal vagal complex, *ENS* enteric nervous system, *NTS* nucleus tractus solitaries, *PVN* paraventricular nucleus of the hypothalamus (*m* magnocellular, *p* parvocellular), *SPN* sacral parasympathetic nucleus

pelvic nerves, which are mixed systems. The VN contains 80–90 % of afferent fibers carrying information from the abdominal organs to the brain [4] with the exception of pelvic viscera for which information is transmitted to the sacral (S2–S4) spinal cord by the pelvic nerves with central projections similar to other spinal visceral afferents. The VN carries mainly mechanoreceptor and chemosensory information of the gut. Vagal afferents do not encode painful stimuli but are able to modulate nociceptive processing in the spinal cord and the brain [149]. The VN is described as the sixth sense based on how sensory vagal information is processed in the CNS [210]. The sympathetic nerves contain 50 % afferent fibers. Visceral afferents that enter via spinal nerves (i.e., splanchnic and pelvic nerves), at thoracic five–lumbar two segments of the spinal cord, carry information concerning temperature as well as nociceptive visceral inputs related to mechanical, chemical, or thermal stimulation through C and Aδ afferents, which will reach conscious perception. The afferent information of the ANS reaches the CNS at both the spinal cord, for the splanchnic nerves, and the NTS in the dorsal medulla, and sacral parasympathetic (S2–S4) for the VN and pelvic nerves, respectively. At the level of the spinal cord, sympathetic afferents are integrated at the level of laminae I, II outer, V, VII (indirectly), and X, and the information is then projected to the upper level through the spinothalamic and spinoreticular tracts, the dorsal column with projection to the thalamus (ventral posterolateral nucleus, intralaminar nucleus) and the cerebral cortex (insular, anterior cingulate, dorsolateral prefrontal cortex, etc.). At the level of the NTS, vagal afferent information is integrated according to a visceral somatotopy in subnuclei (e.g., medial, commissural, gelatinosus) [3] then projecting to the parabrachial nucleus, in the pons,

according to a viscerotopic organization, which in turn sends projections to numerous structures in the brainstem, hypothalamus, basal forebrain, thalamus, and cerebral cortex [65]. Among the cerebral cortex, the insular cortex acts as a visceral (e.g., GI) cortex through an NTS–parabrachial–thalamocortical pathway according to a viscerotopic map. The insular cortex has connections with the limbic system (bed nucleus of the stria terminalis and CeA) and with the lateral frontal cortical system [165]. The NTS also sends projections to the ventrolateral medulla, the parvocellular part of the PVH, and the amygdala/bed nucleus of the stria terminalis contributing to visceral perception. The NTS receives convergent afferents from both the spinal cord (i.e., laminae I, V, VII, and X) and the VN; some of these afferents can be at the origin of autonomic reflex responses. This convergence is also observed at the level of the parabrachial nucleus and ventrolateral medulla [164], thus arguing for a relationship of pain with visceral sensations. The dorsal vagal complex (DVC), located in the brainstem, in the floor of the fourth ventricle, and the PVH are the best candidates for the action of stress and CRF/Ucns in the brain. The DVC is composed of the NTS, the dorsal motor nucleus of the VN (DMNV), which contains the perikarya of efferent vagal preganglionic neurons, and area postrema (AP), which is a circumventricular organ lying dorsally in the middle third of the rostrocaudal extent of the DVC. The PVH and DVC, as well as limbic structures, contain neurons bearing CRF2a [24] and are known to influence autonomic nervous outflow. The messages coming from the gut are integrated in the central autonomic nervous system which contains brain regions involved in the autonomic, endocrine, motor, and behavioral responses [164]. This brain network can be divided into executive structures, mainly hypothalamic, and coordinating structures, mainly included in the limbic system, and high level control structures, mainly the frontal cortex. The LC and the Barrington nucleus are also important parts of this network. The LC, the largest group of noradrenergic neurons located in the pons, mediates emotional arousal, autonomic, and behavioral responses to stress and attention-related processes through its dense projections to most areas of the cerebral cortex [60]. The Barrington nucleus is ventrolaterally located to the LC and projects to the sacral parasympathetic nucleus to increase the motility of the distal recto-colon through an LC-Barrington nucleus interaction [198]. The central autonomic nervous system, in turn, adapts the response of the digestive tract through the efferent ANS (the VNs, the sacral parasympathetic pelvic nerves, and the splanchnic nerves) through reflex loops which are essentially unconscious or become conscious in pathological conditions. Descending pathways that control somatic as well as visceral pain by modulating at the spinal cord-level visceral informations have been described. These pathways are both inhibitory, thus producing analgesia as represented by projections from the periaqueductal gray to the rostroventral medulla and LC descending fibers to the spinal cord, and facilitatory producing hyperalgesia (rostroventral medulla and OFF and ON cells) [195]. Many of the regions of the CNS that we have described above contain the CRFergic system. Among them, the PVH, Barrington's nucleus/LC, and amygdala are well positioned to participate in the reciprocal brain–gut interactions as it pertains to sensory information from the colon and reflex behavioral and autonomic responses to the viscera.

The circumventricular organs are highly vascularized structures with fenestrated capillaries located around the third and fourth ventricles and characterized by the

lack of a blood–brain barrier; they represent points of communication between the blood, the brain, and the cerebrospinal fluid [17]. They are represented by the subfornical organ, median eminence, pineal gland, area postrema, and organum vasculosum of the lamina terminalis. They are involved in sodium and water balance, cardiovascular regulation, metabolic and energetic balance, immune function, regulation of body temperature, vomiting, and reproduction.

Stress Effect on Gastrointestinal Functions

Motility and Secretion (Fig. 7.3)

Acute stress is currently known to delay gastric emptying and small bowel transit while increasing colonic motility in experimental animals as well as in healthy humans [185, 188]. The effects of stress on gastric emptying are reproduced by central (intracerebroventricular or intracisternal) injections of CRF or Ucns, mainly performed in rodents and dogs, and are prevented by central (intracerebroventricular or intracisternal) injection of either nonselective CRF1/CRF2 antagonists (α-helical CRF9−41, d-Phe12CRF12−41, astressin, astressin-B) or selective CRF2 antagonist (astressin2-B), while selective CRF1 antagonists (CP-154,526, antalarmin, NBI 27914) have no effect, thus arguing that CRF and Ucns act primarily through interaction with CRF2 receptors [185]. In contrast to gastric motor function, few data are available about the effects of stress on small intestinal motility, the role of brain CRF/urocortins and their receptors being often studied in parallel with those observed in the stomach. Central injection of CRF and Ucn 1 induces inhibition of duodenal, small intestinal transit, and propulsive motility in rats and dogs [39, 93]. Central injection of CRF1 preferential agonists such as ovine CRF, rat/human CRF, and Ucn 1 induces increase in colonic transit and defecation [33]. Central injection of Ucn 2 and 3, selective CRF2 agonists, is ineffective in mice at a dose similar to that of CRF [116], while this effect is blocked by central administration of either nonselective CRF1/CRF2 receptor antagonists or selective CRF1 antagonists [33, 115]. In addition, central or peripheral injection of a selective CRF1 antagonist reduces stress-induced increase of colonic transit time. CRF1 invalidated mice have significantly less defecation in an open field test than their wild-type littermates [13, 115, 188]. Central injection of a selective CRF2 antagonist (astressin2-B), at a dose which inhibits the CRF2-induced delay in gastric emptying, is not able to reverse the increase in colonic transit following central injection of CRF in rodents [188], thus confirming that CRF1 is the receptor involved in stress-induced colonic motility disturbances.

Numerous data have established the involvement of peripheral CRF signaling in the modulation of secretory function under stress conditions via activation of both CRF1 and CRF2 receptors and activation of cholinergic enteric neurons, mast cells, and possibly serotonergic pathways [101].

The HPA axis is not involved in stress-induced inhibition of gastric emptying. These effects are mediated by the ANS. The effect of CRF and Ucn 1 is mediated by

the VN and abolished by vagotomy [93, 103, 130]. The effect of Ucn 2 requires the integrity of the sympathetic nervous system and peripheral α-adrenergic receptors while not altered by vagotomy [54]. The effect of stress-induced CRF1-dependent activation of colonic motility is mediated by the parasympathetic nervous system, i.e., the celiac branch of the VN, which innervate the proximal colon, and the sacral parasympathetic fibers, which innervate the distal part of the colon and the rectum [128, 130]. The intrinsic nervous system of the colon, as represented by myenteric cholinergic and nitrergic neurons, is involved in the effector mechanism of parasympathetic activation, as well as the action of serotonin (5-HT), from enterochromaffin cells and/or enteric neuron origin, on 5-HT3 and 5-HT4 receptors. The PVN and LC/Barrington's complex are involved in central CRF1 signaling-induced increase in colonic motor function. Changes in *c-fos* and CRF gene expression in the Barrington nucleus have been reported under acute and chronic stress [86], and CRF-synthesizing neurons in the Barrington nucleus project to the noradrenergic LC as well as to the intermediolateral column of the sacral spinal cord, which contains the sacral parasympathetic nucleus innervating the descending colon [197]. There are also CRF efferent projections directly from the PVH to the LC, thus modulating the activity of LC neurons and integrating autonomic responses in brain by influencing LC neurons [151]. Water avoidance stress activates the PVN and LC/Barrington's nuclei and CRF gene transcription in the PVH, whereas icv injection of the nonselective CRF1/CRF2 antagonist α-helical CRF9−41 reduces Fos expression in these nuclei in correlation with defecation score [33]. In agreement with a role for CRF/CRF1 signaling in the PVN in stress-induced increase in colonic motility, α-helical CRF9−41 injected directly into the PVN blocks partial restraint- and water avoidance-induced stimulation of colonic transit and defecation, and various neurogenic and systemic stressors activate the transcription of the CRF1 receptor gene in the PVN ([31]; 130). CRF increases the firing rate of noradrenergic neurons in the LC and releases noradrenalin into the brain prefrontal cortex, an input of the LC, which results in arousal and anxiogenic behavior [198]. Consequently, CRF/CRF1 signaling pathways in the PVN and LC/BN complex may physiologically regulate the behavioral and autonomic responses to stress that influence colonic function as part of the brain–gut axis [129, 198]. These CRF/CRF1 signaling pathways may play a role in diarrhea-predominant IBS patients with psychic comorbidities such as anxiety and depression [115].

Intestinal Permeability

The digestive tract is characterized by an epithelial monolayer essentially composed of enterocytes and colonocytes in the small bowel and colon, respectively, and to a lesser extent mucin-secreting goblet cells, enteroendocrine cells, and others such as intraepithelial lymphocytes. Its integrity is preserved by both cell polarity and the interactions between the cell adhesion complexes, represented by tight junctions and adherens junctions, with the actin cytoskeleton [79]. In physiological

conditions, a limited quantity of antigens goes through the epithelial barrier, playing an important role in the induction of normal immune tolerance toward foreign food antigens and the resident microbiota [88]. The gut is the largest immune organ with the majority of immune cells present in the mucosa. Consequently, a mucosal barrier function is necessary to separate the luminal compartment from the body proper and control antigen-induced inflammation. Any anatomical or functional damage to the intestinal epithelium may allow access of luminal contents to the mucosa then stimulating an inflammatory response in a susceptible host, as observed in IBD, IBS, and celiac disease. This mucosal barrier function is regulated by neuroendocrine and immune factors; thus, it is not surprising that stress, and the CRFergic system, can have a major impact. In contrast to the effects of stress on GI motility, fewer studies have evaluated its effect on intestinal permeability. However, abnormal intestinal permeability has been described in IBS [40] and IBD [175], two GI disorders where stress has a role in their pathogeny [27, 132]. The influence of stress on mucosal function has been recently recognized in short acute stress, early life trauma, and chronic ongoing psychological stress in rats [174]. Indeed, restraint stress or water avoidance stress performed in Wistar–Kyoto rats, a stress-sensitive strain, induces ion secretion, a decrease in barrier function, and an enhancement of small (e.g., proinflammatory bacterial tripeptides) and large molecules (e.g., horseradish peroxidase, HRP) across the epithelium through the transcellular and paracellular pathways [94, 167]. These effects are reproduced by ip injection of CRF 1h before stress [163] and blunted by ip injection of the specific nonselective CRF receptor antagonist, alpha-helical CRF9–41 as well as by pretreatment of the animals with adrenergic and cholinergic antagonists and a mast cell stabilizer. Acute immobilization stress or iv injection of CRF increases colonic mucin release through cholinergic-, adrenergic-, and mast cell-dependent mechanisms [43]. Immobilization stress induces increase in mast cell protease II and mucine release from colonic explants; these effects are prevented by peripheral administration of alpha-helical CRF9-41 [43]. The effects of acute stress on permeability returned to normal within 24 h. These data argue for a complex mechanism of action involving peripheral CRF pathways, mast cell activation, mucine secretion, and increase of intestinal permeability. Mast cells regulate barrier physiology in normal as well as inflamed intestine in rats and humans [19]. Despite convincing evidence of intestinal expression of CRF-like peptides and receptors and its production by local immunocytes and enteroendocrine cells [47, 185], only recently a role for subepithelial CRF–mast cell loops in the regulation of colonic permeability in human biopsies has been reported [204]. Santos et al. [162] have recently shown that the stress-like peptides CRF and sauvagine stimulated ion secretion and macromolecular permeability in the distal colon of WKY rats in vitro. Epithelial responses were inhibited by both the nonselective CRF receptor antagonist astressin and the mast cell stabilizer doxantrazole and significantly reduced in tissues from Ws/Ws rats. These data argue for the involvement of mucosal/submucosal CRF receptors through mast cell-dependent pathways. Neonatal Sprague–Dawley rats, a strain not normally sensitive to stress in contrast to Wistar–Kyoto rats, separated from their mothers from days 2 to 21 for 4 h per day then housed normally until day 60 and then submitted at day

90 to either water avoidance stress or sham stress for 30 min, present an increase of permeability to macromolecules but not in controls [173]. More recently, CRF2 signals through Src/ERK pathway induce the alteration of cell–cell junctions and the shuttle of p120ctn and Kaiso in the nucleus. In HT-29 cells, this signaling pathway also leads to the remodeling of cell adhesion by the phosphorylation of focal adhesion kinase and a modification of actin cytoskeleton and focal adhesion complexes [58].

Gastrointestinal Inflammation

Numerous data argue for a direct autocrine/paracrine effect of CRF on inflammation. CRF reaches the site of inflammation either through nerve terminals or by local release of the peptide by epithelial, endothelial, or resident immune cells. CRF, Ucns, and their receptors are present in mast cells, mouse splenocytes, lymphocytes, monocytes–macrophages, and TH cells [73]. CRF has an opposite effect at the central and peripheral level. Indeed, central CRF has an anti-inflammatory role through the activation of the HPA axis and the release of glucocorticoids, while peripheral CRF acts as a proinflammatory mediator [147, 205]. CRF signals play a role in augmenting LPS-induced proinflammatory cytokine production by macrophages through CRF1 since these effects are inhibited by antalarmin [1]. Peripheral mechanisms by which CRF and Ucn are involved in the development and progression of colitis are not completely clear. Several factors seem involved: the type of inflammatory stimulus, intestinal cell phenotype, type of CRF receptor, and phase of colitis (acute versus chronic). CRF2 and its specific ligand, Ucn 2, appear to mediate proinflammatory responses in the gut. Indeed, CRF-deficient mice develop substantially reduced local inflammatory responses [68] and have dramatically reduced ileal fluid secretion, epithelial cell damage, and neutrophil transmigration 4 h after intraluminal *C. difficile* toxin A [5]. This effect is counterbalanced by administration of the selective CRF2 antagonist astressin 2B. The CRF system is also involved in the inflammatory response associated with IBD [74]. Colonic biopsies from patients with active UC show significantly increased CRF immunoreactive lamina propria mononuclear cells and macrophages [92]. In UC patients without glucocorticoid treatment, Ucn 1-positive cells and plasma cells increased in proportion to the severity of inflammation but are significantly lower in number in glucocorticoid-treated patients. Ucn 1 mRNA was expressed in lamina propria plasma cells, and both CRF1 and CRF2a mRNAs were also partially coexpressed in these cells and macrophages. Ucn 1 therefore may act as a possible local immune-inflammatory mediator in UC [166]. Ucn 2 mRNA is expressed in normal conditions in the small intestine and colon, while in a rat model of chemically induced colitis, Ucn 2 levels are increased, whereas expression levels of its only identified receptor, CRF2, are decreased. This suggests that Ucn 2 exerts its effects only in part via CRF2 [44]. The endogenous upregulation of Ucn 2 following the local inflammatory process accounts for the downregulation of the respective receptor, being either a causing or

a resulting effect. Exposure of human colonic epithelial cells HT-29 to *C. difficile* toxin A or TNF-alpha induces an increase expression of CRF2 mRNA and protein and stimulation of NCM460 colonocytes overexpressing CRF2a with Ucn 2 results in a time- and concentration-dependent increase in IL8 production as well as to an activation of NF-kappaB and MAP kinase in these cells. In addition, expression of Ucn 2 and CRF2 mRNA was increased in mucosal samples of patients with IBD (CD and UC) and after exposure of human intestinal xenografts to C. difficile toxin A. These data suggest that Ucn 2 has proinflammatory effects in human intestinal cells via CRF2a and may be involved in the pathophysiology of colitis in humans [131]. CRF2-deficient mice develop substantially reduced intestinal inflammation and had lower intestinal mRNA expression of the potent chemoattractant keratinocyte chemokine and MCP 1 when exposed to intraluminal C. difficile toxin A. This effect is mimicked by the selective CRF2 antagonist astressin 2B before toxin A exposure. Only Ucn 2, but not other Ucn, was significantly upregulated by ileal administration of toxin A at 4 h compared with buffer exposure [95]. These data argue for CRF2-mediated intestinal inflammation via the release of proinflammatory mediators at the colonocyte level. La Fleur et al. [99] have shown that, in an experimental model of toxin-induced intestinal inflammation, inhibition of CRF ablated the inflammatory response, while Ucn 2 dsRNA treatment did not modify the inflammatory response to toxin. Gonzales-Rey et al. [71] investigated the potential therapeutic effect of Ucn 1 in a murine model of TNBS-induced colitis and showed that Ucn 1 significantly improved the clinical and histopathological severity of colitis and increased the survival rate of mice with colitis. Importantly, Ucn 1 treatment was effective in established colitis and avoided recurrence of the disease. This work identifies Ucn 1 as a potent anti-inflammatory factor. Few data are available regarding the role of the CRFergic system as a pro- or anti-inflammatory system at the level of the stomach. In the upper GI, CRF2 appears to be the most prominent. Indeed, CRF1- and CRF2-positive cells are present in the oxyntic gland and the submucosal blood vessels. No specific CRF1 is observed in the antrum. CRF2 is present in the gastric glands along with immunoreactive Ucn. Thus, both CRF receptor subtypes are expressed in the upper GI tissues with a distinct pattern and regional differences suggesting a differential function. A paracrine CRF-like circuit is present in human stomach composed of Ucn and its CRF2 receptor compared to the CRF/CRF1 circuit in the human colon. Chatzaki et al. [49] examined the presence of CRF and Ucn transcripts and peptides in human gastric mucosa and the association between CRF and Ucn and H. pylori gastritis. They observed the presence of the Ucn transcript in biopsies obtained by gastroscopy from normal and inflamed gastric mucosa, while the CRF transcript was not detectable. Immunoreactive Ucn was localized in gastric epithelial cells and in inflammatory elements of the surrounding negative for Ucn gastric stroma. The level of immunoreactive Ucn was higher in gastric biopsies from patients with active H. pylori gastritis than in controls. Eradication of H. pylori was followed by a dramatic increase of ir-Ucn levels, while nonresponders to the eradication treatment did not show any significant change in ir-Ucn levels. These data suggest that in human gastric epithelium, Ucn is present and plays an important physiological role, while CRF is absent.

In addition, and in contrast to what has been observed for CRF in ulcerative colitis [92], a highly significant but negative correlation has been found between Ucn levels and gastric inflammation, suggesting that Ucn may exert an anti-inflammatory effect in the gastric mucosa. The Ucn/CRF2 system in the upper gastrointestinal tract plays a completely different role in gastric inflammatory response compared with that of the CRF/CRF1 system in the inflammatory response in the colon. These differences between upper and lower GI tracts, *vis-à-vis* their CRF paracrine circuits, may partially explain the lack of severe chronic inflammatory diseases in the stomach compared with the colon in IBD.

Microbiota

There are 10–100 trillion bacterial cells in our gut, ten times more than the number of somatic and germ cells in our body. The number of species is estimated between 500 and 800, and eighty percent of these species are unculturable [59]. New molecular techniques allow inventory of the gut's resident bacterial species without having to culture them. Bacterial genes outnumber our body's genes by as much as 100–1 [7]. The human microbiota is necessary to the healthy functioning of the GI tract and contributes to the intestinal development, metabolic transformations, and protections against enteric infections [75]. Bacteria in the gut have an important role in the immune response, including inflammation [106]. There is a new concept on the bidirectional communication between the nervous system and commensal, pathogenic, and probiotic organisms, i.e., the microbiota–brain–gut axis where gut microorganism can activate the VN; this activation plays a critical role in mediating effects on the brain and behavior. The VN appears to differentiate between non-pathogenic and potentially pathogenic bacteria even in the absence of overt inflammation and mediates signals that can induce both anxiogenic and anxiolytic effects, depending on the nature of the stimulus [62]. Mice treated orally with Campylobacter jejuni showed vagally mediated activation in the NTS in the absence of intestinal inflammation [70]. This axis is vital for maintaining homeostasis and may be involved in the etiology of several metabolic and mental dysfunctions/disorders. 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 (higher plasma ACTH and corticosterone elevation) was observed in germ-free mice that was reversed by reconstitution of the microbiota; germ-free mice also exhibited reduced brain-derived neurotrophic factor (BDNF) expression levels in the cortex and hippocampus [181]. The exaggerated HPA stress response by germ-free mice was reversed by reconstitution with Bifidobacterium infantis. Thus, commensal microbiota can affect the postnatal development of the HPA stress response in mice. Prevention of intestinal barrier impairment by a probiotic attenuates HPA response to an acute psychological stress in rats [2]. In addition, germ-free mice show reduced anxiety-like behavior in comparison to specific pathogen-free mice, a phenotype accompanied by changes in plasticity-related genes in the

hippocampus and amygdala [136], two key structures in the adaptation to the stress response. The intestinal microbiota influences central (i.e., hippocampal) levels of BDNF, which regulates dendritic architecture and spines, and behavior independently of the ANS, gastrointestinal-specific neurotransmitters, or inflammation [18]. Consumption of a fermented milk product with probiotic for 4 weeks by healthy women altered brain intrinsic connectivity or responses to emotional attention tasks [192]; thus, neuroimaging seems to be an interesting tool to study the microbiome–gut–brain axis. In regular functioning conditions, the intestinal barrier is able to prevent most environmental and external antigens to interact openly with the numerous and versatile elements that compose the mucosal-associated immune system. Stress is well known to induce an increase of intestinal permeability that allows bacteria to cross the epithelial barrier to activate mucosal immune response (Killiaan et al. 1998) and to translocate to secondary lymphoid organs [10], thus stimulating the innate immune system. Stress is able to modify the intestinal microbiota [9]. Indeed, exposure of mice to a social stressor affects the structure of the intestinal microbiota and increases the circulating level of cytokines; this effect is reversed by antibiotics [8]. Changes in the intestinal microbiota reduce resistance to infectious challenge with intestinal pathogens [9]. These data provide evidence for the interplay between stress, the intestinal microbiota, and the immune response. This can in turn have significant impact on the host and affect behavior, visceral sensitivity, and inflammatory susceptibility [52]. The sympathetic nervous system, through the release of catecholamines, e.g., norepinephrine, a stress mediator, stimulates growth of bacteria [111]. 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 [110].

Visceral Sensitivity

Stress increases visceral perception and emotional response to visceral events by a perturbation of the brain–gut axis at its different levels, central (brain and spinal cord), peripheral (gut), and the ANS [100]. Genetic models of depression and anxiety, such as the high-anxiety Wistar–Kyoto rats or Flinders Sensitive Line, rats [139] or deleting CRF1, exhibit a decrease of colonic sensitivity to colonic distension [194] while models overexpressing CRF1 exhibit enhanced response [127]. These data argue for the filiation stress–anxiety–inflammation and visceral hypersensitivity. CRF signaling, both at the central and peripheral level, is a key element involved in this effect. Data argue for an equally important contribution of the peripheral CRF/CRF1 signaling locally expressed in the gut to the GI stress response [101]. Indeed, mast cell degranulation in the colon under stress and peripheral administration of CRF induces visceral hypersensitivity through the release of their mediators (histamine, tryptase, prostaglandin E2, nerve growth factor, CRF, TNF) that can stimulate or sensitize sensory afferents [201].

Among the brain structures involved in stress-induced visceral hypersensitivity, the amygdala, a major extrahypothalamic source of CRF, is a key element. Indeed, an activation of the amygdala is observed in experimental models of somatovisceral and visceral pain [171, 172] as well as experimental models of stress-induced GI disturbances [31–33] and colitis [145] as well as in a model of visceral pain in healthy volunteers [6] and IBS patients [30]. Activation of corticosteroid receptor in the CeA is involved in the induction of anxiety and visceral hypersensitivity [135]. Implants of corticosterone micropellets in the CeA increase anxiety-like behavior as well as visceral hypersensitivity to colonic distension and increased responsiveness of viscera-sensitive lumbosacral spinal neurons that mediate visceromotor reflexes to colorectal distension [134]. Water avoidance stress performed for 7 consecutive days induced visceral hypersensitivity that is abolished by glucocorticoid receptor and mineralocorticoid receptor antagonists in the amygdala. Wistar–Kyoto rats express a greater amount of CRF and CRF1 mRNA in the CeA and PVH [36] and depict colonic hypersensitivity to luminal distension which is reversed by peripheral administration of a CRF1 antagonist as well as into the CeA, thus strengthening the role of CRF1 receptor in the amygdala in visceral hypersensitivity mechanism [90]. CRF neurons in the CeA project directly to the LC and increase the firing rate of LC neurons, thus increasing noradrenaline release in the vast terminal fields of this ascending noradrenergic system. The expression of CRF in the LC is increased in WKY rats, and a selective CRF1 receptor antagonist dampened the activation of LC neurons by colorectal distension and intracisternal CRF in rats [96]. CRF1 and CRF2 in the amygdala mediate opposing effects on nociceptive processing, i.e., pro- and antinociceptive effects of CRF, respectively [89]. Low concentrations of CRF facilitate nociceptive processing in the CeA neurons through CRF1, while higher concentrations of CRF have inhibitory effects through CRF2 receptors, in agreement with the concept that CRF2 receptors serve to dampen or reverse CRF1-initiated responses [185]. These results clarify to some extent the controversial role of CRF in pain modulation. Administration of CRF into the CeA significantly increased the number of abdominal muscle contractions in response to colorectal distension in male Wistar rats; this effect was dampened by injection of the CRF1 antagonist, CP-154526. Colorectal distension increased noradrenaline in the CeA which was further increased by CRF and inhibited by CRF1 antagonist. These data suggest that CRF in the CeA sensitizes visceral nociception via CRF1 with release of noradrenaline [180]. The insular cortex is also a key region of the pain matrix, more particularly involved in visceral pain (i.e., colorectal distension) [6, 30]. Bilateral insular cortex lesions in rats markedly inhibit visceral hypersensitivity induced by chronic stress, thus strengthening its role in the pathophysiology of stress-related visceral hypersensitivity [208]. Chronic stress increases DNA methylation and histone acetylation of genes that regulate visceral pain sensation in the peripheral nervous system of rats. These results have potential therapeutic implications when blocking epigenetic regulatory pathways in specific regions of the spinal cord [84].

A temporary disruption of the gut microbiota in early life induces specific and long-lasting changes in visceral sensitivity in male rats, a hallmark of stress-related

functional disorders of the brain–gut axis such as IBS [138]. Early life adversity is known to induce visceral hypersensitivity through ovarian hormones, specifically estradiol, and signaling within the HPA axis, either through reduced negative feedback or increased facilitation, with specific changes in amygdala-mediated mechanisms. Stress-induced visceral hypersensitivity following maternal separation is transferred across generations, this transfer depending on maternal care [200].

Implication in Functional Digestive Disorders and Inflammatory Bowel Diseases

Functional Digestive Disorders: Irritable Bowel Syndrome (Fig. 7.4)

IBS is the most common functional digestive disorders, with an estimated prevalence rate in the general population of 10–15 % in industrialized countries. IBS is characterized by abdominal pain, bloating, and altered bowel habits without any organic cause [132]. Women have a higher prevalence of symptoms than men. IBS accounts for up to 12 % of visits to primary care doctors and 28 % of visits to

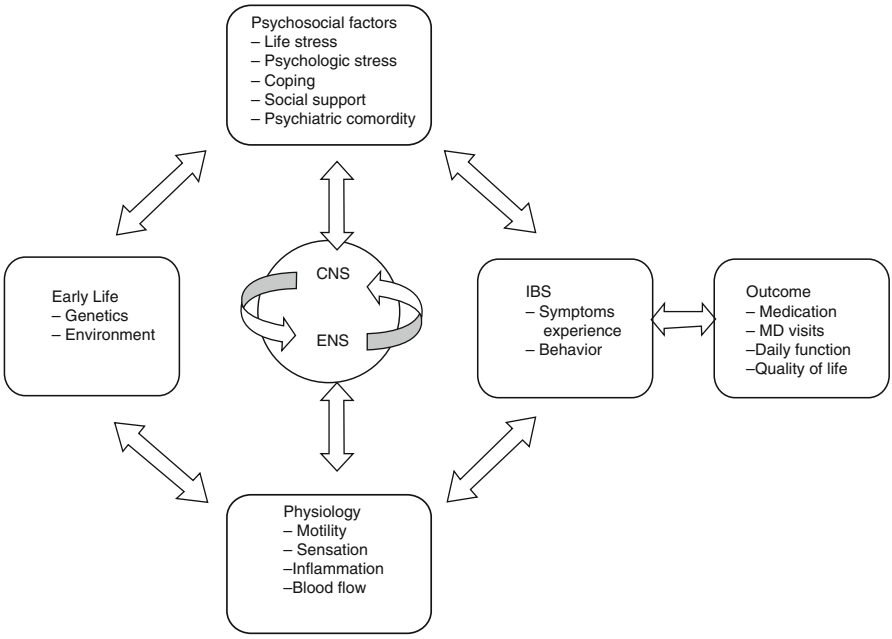

Fig. 7.4 The relationship between early life, psychological factors, physiology, subjective experience of symptoms, behavior, and outcome in irritable bowel syndrome (With permission from Mulak and Bonaz [132])). *CNS* central nervous system, *ENS* enteric nervous system, *IBS* irritable bowel syndrome, *MD* medical doctor

gastroenterologists [41]. IBS is associated with a significant impairment in quality of life, a high rate of absence from work, and a significant increase in healthcare costs. Extraintestinal manifestations are frequently associated to digestive symptoms such as headache, arthralgia, urinary manifestations, insomnia, and fatigue. Fibromyalgia is often observed in IBS and conversely [46]. Psychiatric comorbidity, mainly major depression, anxiety, and somatoform disorders, is observed in 20–50 % of IBS patients [67]; stress is involved in such disorders and psychiatric disorders precede the onset of the GI symptoms [184]. Numerous data argue for a role of stress in the pathophysiology of IBS. Patients with IBS report more stressful life events than medical comparison groups or healthy subjects. Stress is strongly associated with symptom onset and symptom severity in IBS patients. Illness experience, healthcare-seeking behavior, and treatment outcome are adversely affected by stressful life events, chronic social stress, anxiety disorders, and maladaptive coping style. A history of emotional, sexual, or physical abuse is found in 30–50 % of IBS patients [35]. A majority of patients with IBS have a visceral hypersensitivity as represented by lower pain thresholds to intestinal distension compared to healthy controls [153]. Among the peripheral mechanisms of this visceral hypersensitivity, low-grade inflammation in the GI tract could favor modifications of neuronal plasticity [37, 119] and mast cells could also be involved by sensitizing visceral afferent terminals [76]. A postinfectious IBS has been observed in 4–30 % following bacterial gastroenteritis [78]; perceived stress, anxiety, somatization, and negative illness beliefs at the time of infection in favor of a cognitive–behavioral model of IBS were predictors of postinfectious IBS [176]. Modifications in central sensory processing are described in IBS [30]. A spinal hypersensitivity has been evoked in IBS patients as well as a supraspinal cause where stress is of primary importance [132]. A defect of descending pain inhibition pathways is also evoked [206], as described in fibromyalgia patients [102]. Globally, IBS is assimilated to a central sensitization syndrome as observed for chronic fatigue syndrome, fibromyalgia, posttraumatic stress disorders, headaches, restless legs syndrome, and others [209].

Early life traumas, known to increase the risk of developing IBS later in life, play a major role in the development of mood and anxiety disorders and increased CRF signaling [81]. Neonatal maternal separation (MS), as an early life trauma, is a model of IBS in rodents, leading to chronic dysregulation in the limbic–HPA axis [150]. This has led to the biopsychosocial model of IBS [189] and the concept that IBS is due to a brain–gut axis dysfunction consistent with an upregulation in neural processing between the gut and the brain. Globally, there is a hypervigilance state that explains the visceral hypersensitivity observed in these patients most likely through a central (and peripheral) hyper-CRFergic state. In addition, pain is a stress per se that could amplify such disturbances, contributing to its chronicity. Since the ANS is the link between the gut and the brain, it is not surprising in this context of an abnormal brain–gut axis to observe an important dysautonomia, with a high sympathetic and a low parasympathetic tone, whatever the positive or negative affective adjustment [142].

Functional brain imaging studies have allowed a better understanding of the pathophysiology of IBS. Indeed, an abnormal brain processing to visceral pain has been described in IBS patients [30, 193] particularly in brain loci of the pain matrix such as the somatosensory, insular, prefrontal, and cingular cortices as well as sub-

cortical loci such as the thalamus, the amygdala, and the periaqueductal gray. In addition there is a major influence of cognitive–affective processes, including arousal, attention, conditioning and negative affect, and coping strategies, on GI sensations, and its central correlates in health and IBS [118, 142, 202]. The role of the central and/or peripheral CRF system is gaining clinical recognition as part of the neurobiological common denominator of IBS symptoms susceptible to stress and anxiety/depression [63, 186]. Elevated concentrations of CRF in the CSF are observed in patients with anxiety and vulnerability to stress as well as in those suffering from obsessive–compulsive disorders, posttraumatic stress disorders, or childhood trauma, and CRF in the CSF is a predictor of perceived aversive early life experiences [105]. In patients suffering from fibromyalgia, known to have comorbidity with IBS, CSF levels of CRF are associated with both pain symptoms and autonomic dysfunction [122]. An overactivity of the HPA axis and enhanced plasma CRF response to mental stress has been described in IBS patients [146]. IBS patients have previously been proposed to have an exaggerated brain–gut response to CRF [64]. Basal levels of noradrenaline are higher in IBS patients than in controls [82] indicating enhanced activity of the sympathetic nervous system, known to selectively increase visceral sensitivity [87]. An alteration in central noradrenergic signaling is observed in IBS; early life trauma may be one mediator of these abnormalities [20]. In healthy controls, CRF iv decreases rectal pain threshold to distension and mimics an IBS-specific visceral response [137]. Peripheral injection of α-helical CRF9−41 prevents rectal electrical stimulation-induced enhanced sigmoid colonic motility, visceral perception, and anxiety in IBS patients compared to controls without altering the HPA axis [161] and improves decreased alpha power spectra and increases beta power spectra of electroencephalogram in IBS patients [190]. The induction of IBS-like symptoms in healthy subjects and heightened sensitivity in IBS patients are alleviated by a peptide CRF antagonist targeting CRF1 receptor [63] that may provide a new therapeutic avenue in the treatment of IBS [115]. However, in women with diarrhea-predominant IBS, a CRF1 antagonist did not significantly alter colonic or other regional transit or bowel function, thus requiring further study [183].

The medical treatment of IBS is disappointing, often targeting symptoms. In the meantime, because of the complexity of the pathophysiology of IBS, one might wonder that such an ideal treatment would not exist. Nonmedical treatments, as represented by cognitive–behavioral therapy or hypnosis, are of interest [61, 104].

Inflammatory Bowel Diseases (Fig. 7.5)

IBDs are organic diseases classically divided in CD and UC involving the digestive tract, and particularly the small bowel and colon, starting early in life (between 15 and 30 years), and evolving by flares alternating with periods of remissions of variable duration. Symptoms are characterized by abdominal pain, diarrhea, fever, weight loss, and extraintestinal manifestations. The rising incidence of IBD in Western countries supports the hypothesis that “Westernization” of our lifestyle has led to the

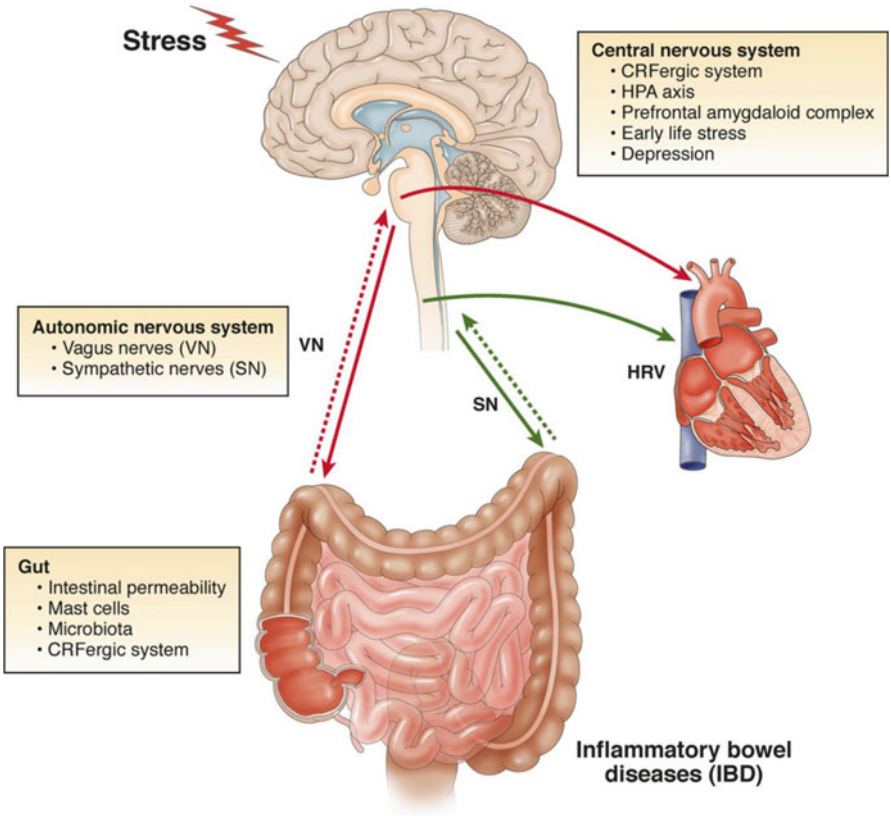

Fig. 7.5 Actors and pathways through which stress may play a role in the pathophysiology of inflammatory bowel disease (With permission from Bonaz and Bernstein [27]). *HRV* heart rate variability, *VN* vagus nerves, *SN* sympathetic nerves

increased incidence of IBD. Today, there is no medical treatment susceptible to cure definitively IBD; the treatment is only suspensive. The pathophysiology of IBD is multifactorial involving immunological, genetic, infectious, and environmental factors [85]. Among the latter, the role of stress is evoked based on experimental and clinical data [27]. IBDs are models of "brain–gut" interactions; they represent an interoceptive (immunogenic) and exteroceptive (psychological) stress involving the neuroendocrine–immune axis. The ANS has a key role in the relation between stress and digestive inflammation. A dysautonomia is reported in IBD, as represented by a sympathetic dysfunction in CD [109] and a vagal dysfunction in UC [108]. This dysautonomia could explain the differential effect of tobacco, nicotine being a parasympathetic activator, which is protective in UC and deleterious in CD. Stress may play a deleterious role in IBD through different pathways close to the ones described for IBS [27] (Fig. 7.5): (1) Activation of mast cells in the intestinal mucosa, in close contact with sympathetic and VN terminals, induces the release of their mediators

(see above) that increase intestinal permeability and activate the mucosal immune function [23, 187]; (2) Catecholamines, acting through α- and β-adrenergic receptors, mediate stress-induced increases in peripheral and central inflammatory cytokines and activation of the inflammatory nuclear factor-kB signaling pathway [91]. Classically, the SNS has a proinflammatory role [120], (3) The VN has a dual anti-inflammatory effect both through its afferents, activating the anti-inflammatory HPA axis, and efferents through the cholinergic anti-inflammatory pathway [28]. Indeed, 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 [140]. VN stimulation (VNS) has been shown to reduce the systemic inflammatory response to endotoxin [34] and intestinal inflammation [57, 124] and could be a nonpharmacological treatment of IBD [28, 51]. The VN also modulates the immune activity of the spleen either directly through connections with the splenic sympathetic nerve [159] or indirectly [114]. Stress has a proinflammatory effect based on its activation of the SNS and adrenomedullary activity while inhibiting the VN [185, 207]. (4) The activity of the sympathovagal balance and the HPA axis, monitored by heart rate variability and cortisol, is linked to the activity of the prefrontal cortex (PFC) and amygdala, respectively [191]. The hypoactivity of the PFC and the enhancement of amygdala activity are strongly influenced by stress [53]. A dysregulation of the amygdala–PFC equilibrium induces an imbalance between the HPA axis and the ANS, as observed in IBD [179], and thus a proinflammatory condition. There is an imbalance between the HPA axis and the vagal tone in CD patients with an inverse association between vagal tone and TNF-alpha level [141], (5) Habituation of the hypothalamic CRFergic system is observed in chronic stress conditions [31]. 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 [98]. A predisposition to a hyporeactive HPA axis and an inhibition of the central response to a chronic interoceptive stress may favor inflammation in IBD, (6) As described above, the CRFergic system is present in the GI tract and may play an anti- or proinflammatory role. 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 favor GI inflammation, (7) There is a microbial basis of IBD [55]. Stress-induced increased intestinal permeability allows bacteria to cross the epithelial barrier to activate mucosal immune response [94] and to translocate to secondary lymphoid organs [10] to stimulate the innate immune system. The sympathetic nervous system, through the release of catecholamines (e.g., norepinephrine), stimulates growth of bacteria [111]. The intestinal microbiota may act as a mediator in the communication between the gut and the brain (i.e., the microbiota brain–gut axis), (8) Modification of the stress axis early in development, as observed in early life traumas, could induce a maladaptive control of neuroendocrine immune axis. Indeed, 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 [169]. Experimental colitis induces a

significantly higher inflammatory reaction in MS animals [14]. Deficient maternal care in rats increases glucocorticoid receptor promoter methylation leading to decreased expression in the hippocampus, a recognized target for glucocorticoid feedback [123]. There is a link between early life stress and depression that may predispose to increased inflammation both under baseline conditions and following stress [56]. MS mice develop a behavioral pattern reminiscent of depression and are more susceptible to inflammation; this vulnerability is reversed by tricyclic antidepressants [203]. Experimental depression in mice is followed by impaired parasympathetic function and increased susceptibility to an experimental colitis that was reduced by desmethylimipramine, through a vagally dependent enhanced parasympathetic function [69]. Most of the data presented above are described in experimental conditions. However, there are now increasing data arguing for a role of stress in IBD patients. 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. In a population-based cohort of IBD patients, significantly more people in the persistently inactive disease group indicated they had experienced no stressful events compared to those in the persistently active disease group. 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 occurrence of a flare [21]. On multivariate logistic regression analyses of these variables, only high perceived stress was associated with increased risk of flare. Perception of stress is a key factor, which incorporates the individual's appraisal of the demands created by stress in general and resources to cope with stress. The interaction between perceived stress and avoidance coping was predictive of earlier relapse in quiescent CD [26]. Chronic stress, including caregiving and marital discord, and perceived stress are associated with increases in CRP and other inflammatory mediators [126]. Stress increased LPS-stimulated cytokines, leukocyte and NK cell counts, platelet activation, and reactive oxygen metabolites production and reduced rectal mucosal blood flow in a study of rectal mucosa of UC patients compared to healthy controls [117]. Sympathetic nerve fibers and neurotransmitters are lost in inflamed areas of the colon in both CD but not in noninflamed ileum [112]; thus, stress may generate symptoms from the uninflamed areas where 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 [72, 148].

At least 33–57 % of IBD patients are able, during the course of their disease, to switch from IBD to IBS [170] most likely through modifications of neuroplasticity induced by chronic inflammation [37]. Proinflammatory cytokines (IL1, IL6, and TNF-α) could favor visceral hyperalgesia via an activation of spinal immune-like glial cells inducing release of proinflammatory substances (IL1, IL6, TNF-α, prostaglandins, nitric oxide) triggering the amplification of pain by modulating the excitability of spinal neurons. However, if visceral hypersensitivity is classically described in IBS, we [160] and others [22, 45] found a visceral hyposensitivity in IBD patients in remission.

Conclusions

Major advances have been made in the domain of stress and CRF signaling pathways in the brain and, more recently, in the gut. The CRFergic system mediates the effects of psychological, physical, and immunological stressors on hormonal responses, anxiety, mood, feeding behavior, and GI functions. Conclusive experimental data show that activation of brain and colonic CRF1 pathways mimics the features of stress-induced stimulation of colonic motility, defecation/watery diarrhea, intestinal permeability, and visceral hypersensitivity described in pathological conditions such as IBS. In contrast, in the upper gut, the brain and gastric CRF2 signaling systems are more prominently involved in CRF ligands- and stress-related suppression of gastric motor function. CRF2 signaling has proinflammatory properties in the lower GI tract but anti-inflammatory properties in the upper GI tract. Targeting these CRF1/CRF2 signaling pathways by selective antagonists/agonists should be of clinical interest in the domain of IBS and IBD.

References

1. Agelaki S, Tsatsanis C, Gravanis A, Margioris AN. Corticotropin-releasing hormone augments proinflammatory cytokine production from macrophages in vitro and in lipopolysaccharide-induced endotoxin shock in mice. Infect Immun. 2002;70(11):6068–74.
2. Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, Houdeau E, Fioramonti J, Bueno L, Theodorou V. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology. 2012;37(11):1885–95.
3. Altschuler SM, Escardo J, Lynn RB, Miselis RR. The central organization of the vagus nerve innervating the colon of the rat. Gastroenterology. 1993;104(2):502–9.
4. Altschuler SM, Bao XM, Bieger D, Hopkins DA, Miselis RR. Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. J Comp Neurol. 1989;283(2):248–68.
5. Anton PM, Gay J, Mykoniatis A, Pan A, O'Brien M, Brown D, Karalis K, Pothoulakis C. Corticotropin-releasing hormone (CRH) requirement in Clostridium difficile toxin A-mediated intestinal inflammation. Proc Natl Acad Sci U S A. 2004;101(22):8503–8.
6. Baciu MV, Bonaz BL, Papillon E, Bost RA, Le Bas JF, Fournet J, Segebarth CM. Central processing of rectal pain: a functional MR imaging study. AJNR Am J Neuroradiol. 1999; 20(10):1920–4.
7. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915–20.
8. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav Immun. 2011;25(3):397–407.
9. Bailey MT, Dowd SE, Parry NM, Galley JD, Schauer DB, Lyte M. Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. Infect Immun. 2010;78(4):1509–19.
10. Bailey MT, Engler H, Sheridan JF. Stress induces the translocation of cutaneous and gastrointestinal microflora to secondary lymphoid organs of C57BL/6 mice. J Neuroimmunol. 2006;171(1–2):29–37.

11. Bale TL. Sensitivity to stress: dysregulation of CRF pathways and disease development. Horm Behav. 2005;48(1):1–10.
12. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. Annu Rev Pharmacol Toxicol. 2004;44:525–57.
13. Bale TL, Lee KF, Vale WW. The role of corticotropin-releasing factor receptors in stress and anxiety. Integr Comp Biol. 2002;42(3):552–5.
14. Barreau F, Ferrier L, Fioramonti J, Bueno L. Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier and mucosal immunity in rats. Gut. 2004;53 (4):501–6.
15. Beaumont W. Nutrition classics. Experiments and observations on the gastric juice and the physiology of digestion. By William Beaumont. Plattsburgh. Printed by F. P. Allen. 1833. Nutr Rev. 1977;35(6):144–5.
16. Behan DP, De Souza EB, Lowry PJ, Potter E, Sawchenko P, Vale WW. Corticotropin releasing factor (CRF) binding protein: a novel regulator of CRF and related peptides. Front Neuroendocrinol. 1995;16(4):362–82.
17. Benarroch EE. Circumventricular organs: receptive and homeostatic functions and clinical implications. Neurology. 2011;77(12):1198–204. doi:10.1212/WNL.0b013e31822f04a0.
18. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, Collins SM. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141(2):599–609, 609.e1-3.
19. Berin MC, Kiliaan AJ, Yang PC, Groot JA, Kitamura Y, Perdue MH. The influence of mast cells on pathways of transepithelial antigen transport in rat intestine. J Immunol. 1998;161(5):2561–6.
20. Berman S, Suyenobu B, Naliboff BD, Bueller J, Stains J, Wong H, Mandelkern M, Fitzgerald L, Ohning G, Gupta A, Labus JS, Tillisch K, Mayer EA. Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome. Neuroimage. 2012;63(4):1854–63. doi:10.1016/j.neuroimage.2012.08.028. Epub 2012 Aug 21.
21. Bernstein CN, Singh S, Graff LA, Walker JR, Miller N, Cheang M. A prospective population-based study of triggers of symptomatic flares in IBD. Am J Gastroenterol. 2010;105(9): 1994–2002.
22. Bernstein CN, Niazi N, Robert M, Mertz H, Kodner A, Munakata J, Naliboff B, Mayer EA. Rectal afferent function in patients with inflammatory and functional intestinal disorders. Pain. 1996;66(2–3):151–61.
23. Bischoff SC. Physiological and pathophysiological functions of intestinal mast cells. Semin Immunopathol. 2009;31(2):185–205.
24. Bittencourt JC, Sawchenko PE. Do centrally administered neuropeptides access cognate receptors?: an analysis in the central corticotropin-releasing factor system. J Neurosci. 2000;20(3):1142–56.
25. Bittencourt JC, Vaughan J, Arias C, Rissman RA, Vale WW, Sawchenko PE. Urocortin expression in rat brain: evidence against a pervasive relationship of urocortin-containing projections with targets bearing type 2 CRF receptors. J Comp Neurol. 1999;415(3):285–312.
26. Bitton A, Dobkin PL, Edwardes MD, Sewitch MJ, Meddings JB, Rawal S, Cohen A, Vermeire S, Dufresne L, Franchimont D, Wild GE. Predicting relapse in Crohn's disease: a biopsychosocial model. Gut. 2008;57(10):1386–92.
27. Bonaz BL, Bernstein CN. Brain-gut interactions in inflammatory bowel disease. Gastroenterology. 2013;144(1):36–49.
28. Bonaz B, Picq C, Sinniger V, Mayol JF, Clarençon D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. Neurogastroenterol Motil. 2013;25 (3):208–21.
29. Bonaz B, Pellissier S, Sinniger V, Clarençon D, Peinnequin A, Canini F. The irritable bowel syndrome: how stress can affect the amygdala activity and the brain-gut axis. In: Ferry DB, editor. The amygdala – a discrete multitasking manager. Rijeka, Croatie: InTech; 2012. p. 339–74.

30. Bonaz B, Baciu M, Papillon E, Bost R, Gueddah N, Le Bas JF, Fournet J, Segebarth C. Central processing of rectal pain in patients with irritable bowel syndrome: an fMRI study. Am J Gastroenterol. 2002;97(3):654–61.
31. Bonaz B, Rivest S. Effect of a chronic stress on CRF neuronal activity and expression of its type 1 receptor in the rat brain. Am J Physiol. 1998;275(5 Pt 2):R1438–49.
32. Bonaz B, Taché Y. Corticotropin-releasing factor and systemic capsaicin-sensitive afferents are involved in abdominal surgery-induced Fos expression in the paraventricular nucleus of the hypothalamus. Brain Res. 1997;748(1–2):12–20.
33. Bonaz B, Taché Y. Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. Brain Res. 1994;641(1):21–8.
34. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405(6785):458–62.
35. Bradford K, Shih W, Videlock EJ, Presson AP, Naliboff BD, Mayer EA, Chang L. Association between early adverse life events and irritable bowel syndrome. Clin Gastroenterol Hepatol. 2012;10(4):385–90.e1-3.
36. Bravo JA, Dinan TG, Cryan JF. Alterations in the central CRF system of two different rat models of comorbid depression and functional gastrointestinal disorders. Int J Neuropsychopharmacol. 2011;14(5):666–83.
37. Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. Nat Rev Gastroenterol Hepatol. 2014;11(10):611–27.
38. Buckinx R, Adriaensen D, Nassauw LV, Timmermans JP. Corticotrophin-releasing factor, related peptides, and receptors in the normal and inflamed gastrointestinal tract. Front Neurosci. 2011;5:54.
39. Bueno L, Fioramonti J. Effects of corticotropin-releasing factor, corticotropin and cortisol on gastrointestinal motility in dogs. Peptides. 1986;7(1):73–7.
40. Camilleri M, Gorman H. Intestinal permeability and irritable bowel syndrome. Neurogastroenterol Motil. 2007;19(7):545–52.
41. Camilleri M. Pathophysiology in irritable bowel syndrome. Drug News Perspect. 2001;14(5):268–78.
42. Cannon WB. The movements of the intestines studied by means of the Röntgen Rays. J Med Res. 1902;7(1):72–5.
43. Castagliuolo I, Lamont JT, Qiu B, Fleming SM, Bhaskar KR, Nikulasson ST, Kornetsky C, Pothoulakis C. Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. Am J Physiol. 1996;271(5 Pt 1):G884–92.
44. Chang J, Hoy JJ, Idumalla PS, Clifton MS, Pecoraro NC, Bhargava A. Urocortin 2 expression in the rat gastrointestinal tract under basal conditions and in chemical colitis. Peptides. 2007;28(7):1453–60.
45. Chang L, Munakata J, Mayer EA, Schmulson MJ, Johnson TD, Bernstein CN, Saba L, Naliboff B, Anton PA, Matin K. Perceptual responses in patients with inflammatory and functional bowel disease. Gut. 2000;47(4):497–505.
46. Chang L. The association of functional gastrointestinal disorders and fibromyalgia. Eur J Surg Suppl. 1998;583:32–6.
47. Chatzaki E, Murphy BJ, Wang L, Million M, Ohning GV, Crowe PD, Petroski R, Taché Y, Grigoriadis DE. Differential profile of CRF receptor distribution in the rat stomach and duodenum assessed by newly developed CRF receptor antibodies. J Neurochem. 2004;88(1):1–11.
48. Chatzaki E, Crowe PD, Wang L, Million M, Taché Y, Grigoriadis DE. CRF receptor type 1 and 2 expression and anatomical distribution in the rat colon. J Neurochem. 2004;90(2):309–16.
49. Chatzaki E, Charalampopoulos I, Leontidis C, Mouzas IA, Tzardi M, Tsatsanis C, Margioris AN, Gravanis A. Urocortin in human gastric mucosa: relationship to inflammatory activity. J Clin Endocrinol Metab. 2003;88(1):478–83.
50. Chen C. Recent advances in small molecule antagonists of the corticotropin-releasing factor type-1 receptor-focus on pharmacology and pharmacokinetics. Curr Med Chem. 2006;13 (11):1261–82.

51. Clarençon D, Pellissier S, Sinniger V, Kibleur A, Hoffman D, Vercueil L, David O, Bonaz B. Long term effects of low frequency (10 hz) vagus nerve stimulation on EEG and heart rate variability in Crohn's disease: a case report. Brain Stimul. 2014;7(6):914–6.
52. Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology. 2009;136(6): 2003–14.
53. Czéh B, Perez-Cruz C, Fuchs E, Flügge G. Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: does hemisphere location matter? Behav Brain Res. 2008;190(1):1–13.
54. Czimmer J, Million M, Taché Y. Urocortin 2 acts centrally to delay gastric emptying through sympathetic pathways while CRF and urocortin 1 inhibitory actions are vagal dependent in rats. Am J Physiol Gastrointest Liver Physiol. 2006;290(3):G511–8. Epub 2005 Oct 13.
55. Dalal SR, Chang EB. The microbial basis of inflammatory bowel diseases. J Clin Invest. 2014;124(10):4190–6.
56. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. Proc Natl Acad Sci U S A. 2007;104(4):1319–24.
57. de Jonge WJ, van der Zanden EP, The FO, Bijlsma MF, van Westerloo DJ, Bennink RJ, Berthoud HR, Uematsu S, Akira S, van den Wijngaard RM, Boeckxstaens GE. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat Immunol. 2005;6(8):844–51.
58. Ducarouge B, Pelissier-Rota M, Lainé M, Cristina N, Vachez Y, Scoazec JY, Bonaz B, Jacquier-Sarlin M. CRF2 signaling is a novel regulator of cellular adhesion and migration in colorectal cancer cells. PLoS One. 2013;8(11):e79335.
59. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005;308 (5728):1635–8.
60. Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. Physiol Rev. 1983;63(3):844–914.
61. Ford AC, Quigley EM, Lacy BE, Lembo AJ, Saito YA, Schiller LR, Soffer EE, Spiegel BM, Moayyedi P. Effect of antidepressants and psychological therapies, including hypnotherapy, in irritable bowel syndrome: systematic review and meta-analysis. Am J Gastroenterol. 2014; 109(9):1350–65.
62. Forsythe P, Bienenstock J, Kunze WA. Vagal pathways for microbiome-brain-gut axis communication. Adv Exp Med Biol. 2014;817:115–33.
63. Fukudo S. Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. J Gastroenterol. 2007;42 Suppl 17:48–51.
64. Fukudo S, Nomura T, Hongo M. Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotropic hormone in normal controls and patients with irritable bowel syndrome. Gut. 1998;42(6):845–9.
65. Fulwiler CE, Saper CB. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res. 1984;319(3):229–59.
66. Furness JB. The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol. 2012;9(5):286–94.
67. Garakani A, Win T, Virk S, Gupta S, Kaplan D, Masand PS. Comorbidity of irritable bowel syndrome in psychiatric patients: a review. Am J Ther. 2003;10(1):61–7.
68. Gay J, Kokkotou E, O'Brien M, Pothoulakis C, Karalis KP. Corticotropin-releasing hormone deficiency is associated with reduced local inflammation in a mouse model of experimental colitis. Endocrinology. 2008;149(7):3403–9. doi:10.1210/en.2007-1703.
69. Ghia JE, Blennerhassett P, Collins SM. Impaired parasympathetic function increases susceptibility to inflammatory bowel disease in a mouse model of depression. J Clin Invest. 2008; 118(6):2209–18.
70. Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N, Lyte M. Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni. Brain Behav Immun. 2005;19(4):334–44.

71. Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Delgado M. Therapeutic effect of urocortin and adrenomedullin in a murine model of Crohn's disease. Gut. 2006;55(6):824–32.
72. Graff LA, Walker JR, Bernstein CN. Depression and anxiety in inflammatory bowel disease: a review of comorbidity and management. Inflamm Bowel Dis. 2009;15(7):1105–18.
73. Gravanis A, Margioris AN. The corticotropin-releasing factor (CRF) family of neuropeptides in inflammation: potential therapeutic applications. Curr Med Chem. 2005;12(13): 1503–12.
74. Gross KJ, Pothoulakis C. Role of neuropeptides in inflammatory bowel disease. Inflamm Bowel Dis. 2007;13(7):918–32.
75. Guarner F. Enteric flora in health and disease. Digestion. 2006;73 Suppl 1:5–12.
76. Gué M, Del Rio-Lacheze C, Eutamene H, Théodorou V, Fioramonti J, Buéno L. Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. Neurogastroenterol Motil. 1997;9(4):271–9.
77. Guillemin R, Rosenberg B. Humoral hypothalamic control of anterior pituitary: a study with combined tissue cultures. Endocrinology. 1955;57(5):599–607.
78. Gwee KA, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, Read NW. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. Lancet. 1996;347(8995):150–3.
79. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta. 2008;1778(3):660–9.
80. Hauger RL, Risbrough V, Brauns O, Dautzenberg FM. Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. CNS Neurol Disord Drug Targets. 2006;5(4):453–79.
81. Heim C, Newport DJ, Miller AH, Nemeroff CB. Long-term neuroendocrine effects of childhood maltreatment. JAMA. 2000;284(18):2321.
82. Heitkemper M, Jarrett M, Cain K, Shaver J, Bond E, Woods NF, Walker E. Increased urine catecholamines and cortisol in women with irritable bowel syndrome. Am J Gastroenterol. 1996;91(5):906–13.
83. Hillhouse EW, Grammatopoulos DK. The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr Rev. 2006;27(3):260–86.
84. Hong S, Zheng G, Wiley JW. Epigenetic regulation of genes that modulate chronic stress-induced visceral pain in the peripheral nervous system. Gastroenterology. 2015;148(1):148–157.e7.
85. Hugot JP, Zouali H, Lesage S, Thomas G. Etiology of the inflammatory bowel diseases. Int J Colorectal Dis. 1999;14(1):2–9.
86. Imaki T, Shibasaki T, Wang XQ, Demura H. Intracerebroventricular administration of corticotropin-releasing factor antagonist attenuates c-fos mRNA expression in the paraventricular nucleus after stress. Neuroendocrinology. 1995;61(4):445–52.
87. Iovino P, Azpiroz F, Domingo E, Malagelada JR. The sympathetic nervous system modulates perception and reflex responses to gut distention in humans. Gastroenterology. 1995;108 (3):680–6.
88. Iweala OI, Nagler CR. Immune privilege in the gut: the establishment and maintenance of non-responsiveness to dietary antigens and commensal flora. Immunol Rev. 2006;213: 82–100.
89. Ji G, Neugebauer V. Differential effects of CRF1 and CRF2 receptor antagonists on pain-related sensitization of neurons in the central nucleus of the amygdala. J Neurophysiol. 2007;97(6):3893–904.
90. Johnson AC, Tran L, Schulkin J, Greenwood-Van Meerveld B. Importance of stress receptor-mediated mechanisms in the amygdala on visceral pain perception in an intrinsically anxious rat. Neurogastroenterol Motil. 2012;24(5):479–86, e219.
91. Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, Fleshner M. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. Neuroscience. 2005;135(4):1295–307.

92. Kawahito Y, Sano H, Mukai S, Asai K, Kimura S, Yamamura Y, Kato H, Chrousos GP, Wilder RL, Kondo M. Corticotropin releasing hormone in colonic mucosa in patients with ulcerative colitis. Gut. 1995;37(4):544–51.
93. Kihara N, Fujimura M, Yamamoto I, Itoh E, Inui A, Fujimiya M. Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats. Am J Physiol Gastrointest Liver Physiol. 2001;280(3):G406–19.
94. Kiliaan AJ, Saunders PR, Bijlsma PB, Berin MC, Taminiau JA, Groot JA, Perdue MH. Stress stimulates transepithelial macromolecular uptake in rat jejunum. Am J Physiol. 1998;275(5 Pt 1):G1037–44.
95. Kokkotou E, Torres D, Moss AC, O'Brien M, Grigoriadis DE, Karalis K, Pothoulakis C. Corticotropin-releasing hormone receptor 2-deficient mice have reduced intestinal inflammatory responses. J Immunol. 2006;177(5):3355–61.
96. Kosoyan HP, Grigoriadis DE, Taché Y. The CRF(1) receptor antagonist, NBI-35965, abolished the activation of locus coeruleus neurons induced by colorectal distension and intracisternal CRF in rats. Brain Res. 2005;1056(1):85–96.
97. Kozicz T, Yanaihara H, Arimura A. Distribution of urocortin-like immunoreactivity in the central nervous system of the rat. J Comp Neurol. 1998;391(1):1–10.
98. Kresse AE, Million M, Saperas E, Taché Y. Colitis induces CRF expression in hypothalamic magnocellular neurons and blunts CRF gene response to stress in rats. Am J Physiol Gastrointest Liver Physiol. 2001;281(5):G1203–13.
99. la Fleur SE, Wick EC, Idumalla PS, Grady EF, Bhargava A. Role of peripheral corticotropin-releasing factor and urocortin II in intestinal inflammation and motility in terminal ileum. Proc Natl Acad Sci U S A. 2005;102(21):7647–52.
100. Larauche M, Mulak A, Taché Y. Stress and visceral pain: from animal models to clinical therapies. Exp Neurol. 2012;233(1):49–67.
101. Larauche M, Kiank C, Tache Y. Corticotropin releasing factor signaling in colon and ileum: regulation by stress and pathophysiological implications. J Physiol Pharmacol. 2009;60 Suppl 7:33–46.
102. Lautenbacher S, Rollman GB. Possible deficiencies of pain modulation in fibromyalgia. Clin J Pain. 1997;13(3):189–96.
103. Lee C, Sarna SK. Central regulation of gastric emptying of solid nutrient meals by corticotropin releasing factor. Neurogastroenterol Motil. 1997;9(4):221–9.
104. Lee HH, Choi YY, Choi MG. The efficacy of hypnotherapy in the treatment of irritable bowel syndrome: a systematic review and meta-analysis. J Neurogastroenterol Motil. 2014;20 (2):152–62.
105. Lee R, Geracioti Jr TD, Kasckow JW, Coccaro EF. Childhood trauma and personality disorder: positive correlation with adult CSF corticotropin-releasing factor concentrations. Am J Psychiatry. 2005;162(5):995–7.
106. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science. 2010;330(6012):1768–73.
107. Li C, Vaughan J, Sawchenko PE, Vale WW. Urocortin III-immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. J Neurosci. 2002;22(3):991–1001.
108. Lindgren S, Stewenius J, Sjölund K, Lilja B, Sundkvist G. Autonomic vagal nerve dysfunction in patients with ulcerative colitis. Scand J Gastroenterol. 1993;28(7):638–42.
109. Lindgren S, Lilja B, Rosén I, Sundkvist G. Disturbed autonomic nerve function in patients with Crohn's disease. Scand J Gastroenterol. 1991;26(4):361–6.
110. Lyte M, Vulchanova L, Brown DR. Stress at the intestinal surface: catecholamines and mucosa-bacteria interactions. Cell Tissue Res. 2011;343(1):23–32.
111. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. Bioessays. 2011;33(8):574–81.
112. Magro F, Vieira-Coelho MA, Fraga S, Serrão MP, Veloso FT, Ribeiro T, Soares-da-Silva P. Impaired synthesis or cellular storage of norepinephrine, dopamine, and 5-hydroxytryptamine in human inflammatory bowel disease. Dig Dis Sci. 2002;47(1):216–24.

113. Mano-Otagiri A, Shibasaki T. Distribution of urocortin 2 and urocortin 3 in rat brain. J Nippon Med Sch. 2004;71(6):358–9.
114. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. Auton Neurosci. 2014;182:65–9.
115. Martinez V, Taché Y. CRF1 receptors as a therapeutic target for irritable bowel syndrome. Curr Pharm Des. 2006;12(31):4071–88.
116. Martinez V, Wang L, Million M, Rivier J, Taché Y. Urocortins and the regulation of gastrointestinal motor function and visceral pain. Peptides. 2004;25(10):1733–44.
117. Mawdsley JE, Macey MG, Feakins RM, Langmead L, Rampton DS. The effect of acute psychologic stress on systemic and rectal mucosal measures of inflammation in ulcerative colitis. Gastroenterology. 2006;131(2):410–9.
118. Mayer EA, Naliboff BD, Chang L. Basic pathophysiologic mechanisms in irritable bowel syndrome. Dig Dis. 2001;19(3):212–8.
119. Mayer EA, Gebhart GF. Basic and clinical aspects of visceral hyperalgesia. Gastroenterology. 1994;107(1):271–93.
120. McCafferty DM, Wallace JL, Sharkey KA. Effects of chemical sympathectomy and sensory nerve ablation on experimental colitis in the rat. Am J Physiol. 1997;272(2 Pt 1):G272–80.
121. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Horm Behav. 2003;43(1):2–15.
122. McLean SA, Williams DA, Stein PK, Harris RE, Lyden AK, Whalen G, Park KM, Liberzon I, Sen A, Gracely RH, Baraniuk JN, Clauw DJ. Cerebrospinal fluid corticotropin-releasing factor concentration is associated with pain but not fatigue symptoms in patients with fibromyalgia. Neuropsychopharmacology. 2006;31(12):2776–82.
123. Meaney MJ, Szyf M, Seckl JR. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med. 2007;13(7):269–77.
124. Meregnani J, Clarençon D, Vivier M, Peinnequin A, Mouret C, Sinniger V, Picq C, Job A, Canini F, Jacquier-Sarlin M, Bonaz B. Anti-inflammatory effect of vagus nerve stimulation in a rat model of inflammatory bowel disease. Auton Neurosci. 2011;160(1–2):82–9.
125. Miampamba M, Maillot C, Million M, Taché Y. Peripheral CRF activates myenteric neurons in the proximal colon through CRF(1) receptor in conscious rats. Am J Physiol Gastrointest Liver Physiol. 2002;282(5):G857–65.
126. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65(9):732–41.
127. Million M, Wang L, Stenzel-Poore MP, Coste SC, Yuan PQ, Lamy C, Rivier J, Buffington T, Taché Y. Enhanced pelvic responses to stressors in female CRF-overexpressing mice. Am J Physiol Regul Integr Comp Physiol. 2007;292(4):R1429–38.
128. Million M, Wang L, Martinez V, Taché Y. Differential Fos expression in the paraventricular nucleus of the hypothalamus, sacral parasympathetic nucleus and colonic motor response to water avoidance stress in Fischer and Lewis rats. Brain Res. 2000;877(2):345–53.
129. Mönnikes H, Schmidt BG, Tebbe J, Bauer C, Taché Y. Microinfusion of corticotropin releasing factor into the locus coeruleus/subcoeruleus nuclei stimulates colonic motor function in rats. Brain Res. 1994;644(1):101–8.
130. Mönnikes H, Schmidt BG, Raybould HE, Taché Y. CRF in the paraventricular nucleus mediates gastric and colonic motor response to restraint stress. Am J Physiol. 1992;262(1 Pt 1):G137–43.
131. Moss AC, Anton P, Savidge T, Newman P, Cheifetz AS, Gay J, Paraschos S, Winter MW, Moyer MP, Karalis K, Kokkotou E, Pothoulakis C. Urocortin II mediates pro-inflammatory effects in human colonocytes via corticotropin-releasing hormone receptor 2alpha. Gut. 2007;56(9):1210–7.
132. Mulak A, Bonaz B. Irritable bowel syndrome: a model of the brain-gut interactions. Med Sci Monit. 2004;10(4):RA55–62.
133. Muramatsu Y, Fukushima K, Iino K, Totsune K, Takahashi K, Suzuki T, Hirasawa G, Takeyama J, Ito M, Nose M, Tashiro A, Hongo M, Oki Y, Nagura H, Sasano H. Urocortin and

corticotropin-releasing factor receptor expression in the human colonic mucosa. Peptides. 2000;21(12):1799–809.
134. Myers B, Dittmeyer K, Greenwood-Van Meerveld B. Involvement of amygdaloid corticosterone in altered visceral and somatic sensation. Behav Brain Res. 2007;181(1):163–7.
135. Myers B, Greenwood-Van Meerveld B. Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. Am J Physiol Gastrointest Liver Physiol. 2007;292(6):G1622–9.
136. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil. 2011;23(3):255–64, e119.
137. Nozu T, Kudaira M. Corticotropin-releasing factor induces rectal hypersensitivity after repetitive painful rectal distention in healthy humans. J Gastroenterol. 2006;41(8):740–4.
138. O'Mahony SM, Felice VD, Nally K, Savignac HM, Claesson MJ, Scully P, Woznicki J, Hyland NP, Shanahan F, Quigley EM, Marchesi JR, O'Toole PW, Dinan TG, Cryan JF. Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. Neuroscience. 2014;277:885–901.
139. Overstreet DH, Djuric V. A genetic rat model of cholinergic hypersensitivity: implications for chemical intolerance, chronic fatigue, and asthma. Ann N Y Acad Sci. 2001;933:92–102.
140. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med. 2003;9(5–8):125–34.
141. Pellissier S, Dantzer C, Mondillon L, Trocme C, Gauchez AS, Ducros V, Mathieu N, Toussaint B, Fournier A, Canini F, Bonaz B. Relationship between vagal tone, cortisol, TNF-alpha, epinephrine and negative affects in Crohn's disease and irritable bowel syndrome. PLoS One. 2014;9(9):e105328.
142. Pellissier S, Dantzer C, Canini F, Mathieu N, Bonaz B. Psychological adjustment and autonomic disturbances in inflammatory bowel diseases and irritable bowel syndrome. Psychoneuroendocrinology. 2010;35(5):653–62.
143. Porcher C, Peinnequin A, Pellissier S, Meregnani J, Sinniger V, Canini F, Bonaz B. Endogenous expression and in vitro study of CRF-related peptides and CRF receptors in the rat gastric antrum. Peptides. 2006;27(6):1464–75.
144. Porcher C, Juhem A, Peinnequin A, Sinniger V, Bonaz B. Expression and effects of metabotropic CRF1 and CRF2 receptors in rat small intestine. Am J Physiol Gastrointest Liver Physiol. 2005;288(5):G1091–103.
145. Porcher C, Sinniger V, Juhem A, Mouchet P, Bonaz B. Neuronal activity and CRF receptor gene transcription in the brains of rats with colitis. Am J Physiol Gastrointest Liver Physiol. 2004;287(4):G803–14.
146. Posserud I, Agerforz P, Ekman R, Björnsson ES, Abrahamsson H, Simrén M. Altered visceral perceptual and neuroendocrine response in patients with irritable bowel syndrome during mental stress. Gut. 2004;53(8):1102–8.
147. Radulovic M, Spiess J. Immunomodulatory role of the corticotropin-releasing factor. Arch Immunol Ther Exp (Warsz). 2001;49(1):33–8.
148. Rampton DS. The influence of stress on the development and severity of immune-mediated diseases. J Rheumatol Suppl. 2011;88:43–7.
149. Randich A, Gebhart GF. Vagal afferent modulation of nociception. Brain Res Brain Res Rev. 1992;17(2):77–99.
150. Renard GM, Rivarola MA, Suárez MM. Sexual dimorphism in rats: effects of early maternal separation and variable chronic stress on pituitary-adrenal axis and behavior. Int J Dev Neurosci. 2007;25(6):373–9.
151. Reyes BA, Valentino RJ, Xu G, Van Bockstaele EJ. Hypothalamic projections to locus coeruleus neurons in rat brain. Eur J Neurosci. 2005;22(1):93–106.
152. Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, Hogenesch JB, Gulyas J, Rivier J, Vale WW, Sawchenko PE. Urocortin II: a member of the corticotropin-releasing fac-

tor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc Natl Acad Sci U S A. 2001;98(5):2843–8.
153. Ritchie J. Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. Gut. 1973;14(2):125–32.
154. Rivier CL, Grigoriadis DE, Rivier JE. Role of corticotropin-releasing factor receptors type 1 and 2 in modulating the rat adrenocorticotropin response to stressors. Endocrinology. 2003;144(6):2396–403.
155. Rivier J, Gulyas J, Kunitake K, DiGruccio M, Cantle JP, Perrin MH, Donaldson C, Vaughan J, Million M, Gourcerol G, Adelson DW, Rivier C, Taché Y, Vale W. Stressin1-A, a potent corticotropin releasing factor receptor 1 (CRF1)-selective peptide agonist. J Med Chem. 2007;50(7):1668–74.
156. Rivier J, Gulyas J, Kirby D, Low W, Perrin MH, Kunitake K, DiGruccio M, Vaughan J, Reubi JC, Waser B, Koerber SC, Martinez V, Wang L, Taché Y, Vale W. Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. J Med Chem. 2002;45(21):4737–47.
157. Rivier J, Rivier C, Vale W. Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. Science. 1984;224(4651):889–91.
158. Rivier JE, Kirby DA, Lahrichi SL, Corrigan A, Vale WW, Rivier CL. Constrained corticotropin releasing factor antagonists (astressin analogues) with long duration of action in the rat. J Med Chem. 1999;42(16):3175–82.
159. Rosas-Ballina M, Ochani M, Parrish WR, Ochani K, Harris YT, Huston JM, Chavan S, Tracey KJ. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. Proc Natl Acad Sci U S A. 2008;105(31):11008–13.
160. Rubio A, Pellissier S, Picot A, Dantzer C, Bonaz B. The link between negative affect, vagal tone, and visceral sensitivity in quiescent Crohn's disease. Neurogastroenterol Motil. 2014;26(8):1200–3.
161. Sagami Y, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, Shoji T, Karahashi K, Hongo M, Fukudo S. Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. Gut. 2004;53 (7):958–64.
162. Santos J, Yates D, Guilarte M, Vicario M, Alonso C, Perdue MH. Stress neuropeptides evoke epithelial responses via mast cell activation in the rat colon. Psychoneuroendocrinology. 2008;33(9):1248–56.
163. Santos J, Saunders PR, Hanssen NP, Yang PC, Yates D, Groot JA, Perdue MH. Corticotropin-releasing hormone mimics stress-induced colonic epithelial pathophysiology in the rat. Am J Physiol. 1999;277(2 Pt 1):G391–9.
164. Saper CB. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. Annu Rev Neurosci. 2002;25:433–69.
165. Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. J Comp Neurol. 1982;210(2):163–73.
166. Saruta M, Takahashi K, Suzuki T, Torii A, Kawakami M, Sasano H. Urocortin 1 in colonic mucosa in patients with ulcerative colitis. J Clin Endocrinol Metab. 2004;89(11):5352–61.
167. Saunders PR, Santos J, Hanssen NP, Yates D, Groot JA, Perdue MH. Physical and psychological stress in rats enhances colonic epithelial permeability via peripheral CRH. Dig Dis Sci. 2002;47(1):208–15.
168. Selye H. A syndrome produced by diverse nocuous agents. 1936. J Neuropsychiatry Clin Neurosci. 1998;10(2):230–1.
169. Shanks N, Windle RJ, Perks PA, Harbuz MS, Jessop DS, Ingram CD, Lightman SL. Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. Proc Natl Acad Sci U S A. 2000;97(10):5645–50.
170. Simrén M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Björnsson ES. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol. 2002;97(2):389–96.
171. Sinniger V, Mouchet P, Bonaz B. Effect of nor-trimebutine on neuronal activation induced by a noxious stimulus or an acute colonic inflammation in the rat. Life Sci. 2005;77(23):2927–41.

172. Sinniger V, Porcher C, Mouchet P, Juhem A, Bonaz B. c-fos and CRF receptor gene transcription in the brain of acetic acid-induced somato-visceral pain in rats. Pain. 2004;110(3): 738–50.
173. Söderholm JD, Yates DA, Gareau MG, Yang PC, MacQueen G, Perdue MH. Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. Am J Physiol Gastrointest Liver Physiol. 2002;283(6):G1257–63.
174. Söderholm JD, Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. Am J Physiol Gastrointest Liver Physiol. 2001;280(1):G7–13.
175. Söderholm JD, Peterson KH, Olaison G, Franzén LE, Weström B, Magnusson KE, Sjödahl R. Epithelial permeability to proteins in the noninflamed ileum of Crohn's disease? Gastroenterology. 1999;117(1):65–72.
176. Spence MJ, Moss-Morris R. The cognitive behavioural model of irritable bowel syndrome: a prospective investigation of patients with gastroenteritis. Gut. 2007;56(8):1066–71.
177. Stengel A, Taché Y. CRF and urocortin peptides as modulators of energy balance and feeding behavior during stress. Front Neurosci. 2014;8:52.
178. Stengel A, Taché Y. Corticotropin-releasing factor signaling and visceral response to stress. Exp Biol Med (Maywood). 2010;235(10):1168–78.
179. Straub RH, Herfarth H, Falk W, Andus T, Schölmerich J. Uncoupling of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis in inflammatory bowel disease? J Neuroimmunol. 2002;126(1–2):116–25.
180. Su J, Tanaka Y, Muratsubaki T, Kano M, Kanazawa M, Fukudo S. Injection of corticotropin-releasing hormone into the amygdala aggravates visceral nociception and induces noradrenaline release in rats. Neurogastroenterol Motil. 2015;27(1):30–9.
181. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol. 2004;558(Pt 1):263–75.
182. Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology. 1983;36(3):165–86.
183. Sweetser S, Camilleri M, Linker Nord SJ, Burton DD, Castenada L, Croop R, Tong G, Dockens R, Zinsmeister AR. Do corticotropin releasing factor-1 receptors influence colonic transit and bowel function in women with irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol. 2009;296(6):G1299–306.
184. Sykes MA, Blanchard EB, Lackner J, Keefer L, Krasner S. Psychopathology in irritable bowel syndrome: support for a psychophysiological model. J Behav Med. 2003;26(4):361–72.
185. Taché Y, Bonaz B. Corticotropin-releasing factor receptors and stress-related alterations of gut motor function. J Clin Invest. 2007;117(1):33–40.
186. Taché Y, Martinez V, Wang L, Million M. CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. Br J Pharmacol. 2004;141(8):1321–30.
187. Taché Y, Perdue MH. Role of peripheral CRF signalling pathways in stress-related alterations of gut motility and mucosal function. Neurogastroenterol Motil. 2004;16 Suppl 1:137–42.
188. Taché Y, Martinez V, Million M, Wang L. Stress and the gastrointestinal tract III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors. Am J Physiol Gastrointest Liver Physiol. 2001;280(2):G173–7.
189. Tanaka Y, Kanazawa M, Fukudo S, Drossman DA. Biopsychosocial model of irritable bowel syndrome. J Neurogastroenterol Motil. 2011;17(2):131–9.
190. Tayama J, Sagami Y, Shimada Y, Hongo M, Fukudo S. Effect of alpha-helical CRH on quantitative electroencephalogram in patients with irritable bowel syndrome. Neurogastroenterol Motil. 2007;19(6):471–83.
191. Thayer JF, Sternberg E. Beyond heart rate variability: vagal regulation of allostatic systems. Ann N Y Acad Sci. 2006;1088:361–72.
192. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, Guyonnet D, Legrain-Raspaud S, Trotin B, Naliboff B, Mayer EA. Consumption of fermented milk product with probiotic modulates brain activity. Gastroenterology. 2013;144(7):1394–401. 1401.e1–4.

193. Tillisch K, Mayer EA, Labus JS. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. Gastroenterology. 2011;140(1):91–100.
194. Trimble N, Johnson AC, Foster A, Greenwood-van Meerveld B. Corticotropin-releasing factor receptor 1-deficient mice show decreased anxiety and colonic sensitivity. Neurogastroenterol Motil. 2007;19(9):754–60.
195. Tsuruoka M, Wang D, Tamaki J, Inoue T. Descending influence from the nucleus locus coeruleus/subcoeruleus on visceral nociceptive transmission in the rat spinal cord. Neuroscience. 2010;165(4):1019–24.
196. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science. 1981;213(4514):1394–7.
197. Valentino RJ, Kosboth M, Colflesh M, Miselis RR. Transneuronal labeling from the rat distal colon: anatomic evidence for regulation of distal colon function by a pontine corticotropin-releasing factor system. J Comp Neurol. 2000;417(4):399–414.
198. Valentino RJ, Miselis RR, Pavcovich LA. Pontine regulation of pelvic viscera: pharmacological target for pelvic visceral dysfunctions. Trends Pharmacol Sci. 1999;20(6):253–60.
199. Valentino RJ, Page ME, Luppi PH, Zhu Y, Van Bockstaele E, Aston-Jones G. Evidence for widespread afferents to Barrington's nucleus, a brainstem region rich in corticotropin-releasing hormone neurons. Neuroscience. 1994;62(1):125–43.
200. van den Wijngaard RM, Stanisor OI, van Diest SA, Welting O, Wouters MM, Cailotto C, de Jonge WJ, Boeckxstaens GE. Susceptibility to stress induced visceral hypersensitivity in maternally separated rats is transferred across generations. Neurogastroenterol Motil. 2013; 25(12):e780–90.
201. van den Wijngaard RM, Klooker TK, de Jonge WJ, Boeckxstaens GE. Peripheral relays in stress-induced activation of visceral afferents in the gut. Auton Neurosci. 2010;153 (1–2):99–105.
202. Van Oudenhove L, Coen SJ, Aziz Q. Functional brain imaging of gastrointestinal sensation in health and disease. World J Gastroenterol. 2007;13(25):3438–45.
203. Varghese AK, Verdú EF, Bercik P, Khan WI, Blennerhassett PA, Szechtman H, Collins SM. Antidepressants attenuate increased susceptibility to colitis in a murine model of depression. Gastroenterology. 2006;130(6):1743–53.
204. Wallon C, Yang PC, Keita AV, Ericson AC, McKay DM, Sherman PM, Perdue MH, Söderholm JD. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. Gut. 2008;57(1):50–8.
205. Webster EL, Torpy DJ, Elenkov IJ, Chrousos GP. Corticotropin-releasing hormone and inflammation. Ann N Y Acad Sci. 1998;840:21–32.
206. Willer JC, Bouhassira D, Le Bars D. Neurophysiological bases of the counterirritation phenomenon: diffuse control inhibitors induced by nociceptive stimulation. Neurophysiol Clin. 1999;29(5):379–400.
207. Wood SK, Woods JH. Corticotropin-releasing factor receptor-1: a therapeutic target for cardiac autonomic disturbances. Expert Opin Ther Targets. 2007;11(11):1401–13.
208. Yi L, Sun H, Ge C, Chen Y, Peng H, Jiang Y, Wu P, Tang Y, Meng Q, Xu S. Role of insular cortex in visceral hypersensitivity model in rats subjected to chronic stress. Psychiatry Res. 2014;220(3):1138–43.
209. Yunus MB. Role of central sensitization in symptoms beyond muscle pain, and the evaluation of a patient with widespread pain. Best Pract Res Clin Rheumatol. 2007;21(3):481–97.
210. Zagon A. Does the vagus nerve mediate the sixth sense? Trends Neurosci. 2001;24(11): 671–3.

Chapter 8
Nutrition, Macrobiotics, and the Brain's Neuroinflammatory Response

Violeta Arsenescu

Abstract Environmental factors play an important role in the development of chronic brain inflammatory and neurodegenerative conditions. Diet-derived bioactive molecules can modulate the blood-brain barrier and immune cell traffic to the brain. Alteration in the brain cytokine milieu results in microglial polarization that dictates the outcome of inflammatory process. Preclinical and clinical studies have revealed the anti-inflammatory properties of plant-derived compounds, such as polyphenols and polyunsaturated fatty acids. Nevertheless, no specific diets have been adopted in patients with multiple sclerosis (MS) or Alzheimer's or Parkinson's diseases.

The aim of this review was to provide a framework for nutritional interventions in patients with chronic brain inflammatory and neurodegenerative conditions. The effects of diet are analyzed in the context of complex interaction with gut microbiota and preexistent gut barrier defects as seen in patients with inflammatory bowel disease.

Keywords Nutrition • Neuroinflammation • Multiple sclerosis • Alzheimer • Parkinson • Inflammatory bowel disease • Blood-brain barrier • Gut microbiota • Calprotectin • Microglia • T cells • Aryl hydrocarbon receptor • PUFA • Polyphenols • Autoimmunity

It is known that proteins, fats, and carbohydrates are indispensable food groups for growth and maintenance of life. Besides their nutritive value, new scientific evidence points toward the nonnutritive function of several components of these large food groups. These physiologically active substances and bioactive compounds within the food matrix are known as functional foods and are suggested or expected to provide health benefits. Key substances include flavonoids, antioxidants, vitamins, minerals, fatty acids, phospholipids, and phytochemicals. Functional foods

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may naturally contain these beneficial compounds or may be enriched with/or formulated with these bioactive compounds. Functional foods are distinct from medical foods (formulated to be administered by physicians for disease management) or dietary supplements (non-foods supplementing the conventional food diet). Despite advances in understanding the pathophysiology of chronic diseases, including neurological disorders, we lack adequate treatments that can reverse these chronic illnesses. Currently, there are anti-inflammatory, antioxidant, or anticarcinogenic synthetic drugs that can be used for long-term treatment, but they have multiple side effects. In comparison, functional foods are natural and safe products that can enhance individual health, well-being, and resilience to chronic diseases.

Here, we review the effects of nutrition on the molecular mechanisms that lead to chronic inflammation and degeneration in the central nervous system (Fig. 8.1).

The Brain's Neuroinflammatory Response

The central nervous system (CNS) is an immune-privileged apparatus due to its specialized blood-brain barrier (BBB) composed of astrocytes, endothelial cells, pericytes, and tight junctions [1]. Environmental factors such as trauma, toxins, and diet can modulate an inflammatory response that activates the resident immune cells, microglia. Subsequent recruitment of peripheral blood immune cells leads to a chronic inflammatory and neurodegenerative process [2]. Similar to peripheral macrophages, microglia can be polarized toward the M1 phenotype by lipopolysaccharides (LPS) or interferon gamma (IFN_Y), while interleukin (IL)-4 and IL-13 promote an M2 phenotype [3]. Microglial polarization will alter the local

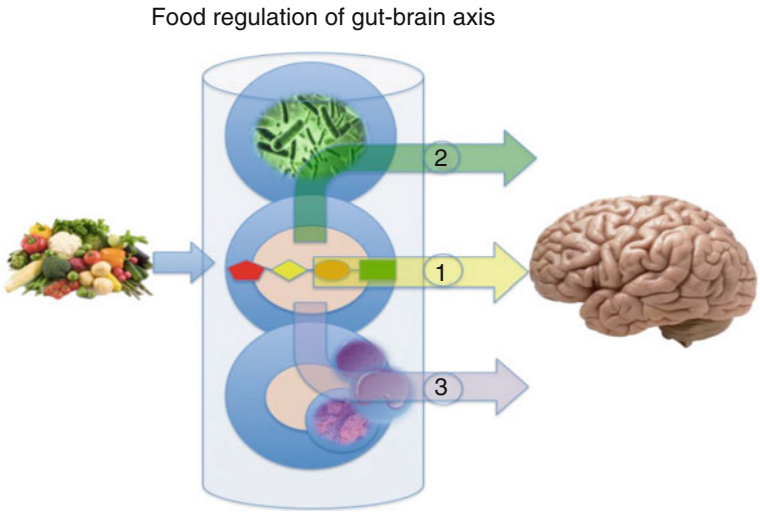

Fig. 8.1 Nutrient pathways that lead to chronic inflammation and degeneration in the central nervous system. (*1*) Dietary metabolites – CNS pathway. (*2*) Diet – gut microbiota – CNS pathway. (*3*) Diet – GALT – CNS pathway

environment and the permeability of the BBB through the production of pro-inflammatory and immunomodulatory cytokines IL-1, IL-6, tumor necrosis factor alpha (TNFα), IL-10, as well as chemokines that enhance the immune traffic [4, 5]. Thus, interventions aimed at restoring the balance between M1 and M2 microglia could alter the course of neurodegenerative processes.

Gut-Centric Pathways Modulate Brain Inflammation

The intestinal barrier is a complex, multilayered structure that regulates nutrient absorption, electrolyte, and water exchanges, as well as the entry of pathogenic microorganisms. The cross talk between gut epithelial cells, intestinal microbiota, and gut-associated immune system provides a dynamic regulatory network that is essential for homeostasis. Gut barrier dysfunction allows abnormal transfer of molecules that result in direct or indirect activation of immune cells at distant sites such as the CNS. Host genetic polymorphisms or preexisting inflammatory responses dictate which remote target will be activated as a consequence of the primary gut barrier defect. On the other hand, even the physiological function of one barrier can influence the other. Stressful events and food allergies can activate the gut resident mast cells via the enteric nervous system. In turn, mast cell degranulation can trigger a systemic inflammatory response that affects the blood-brain barrier. For example, these series of events have been shown as a mechanistic link between autistic disorders and environmental factors.

Inflammatory bowel conditions are associated with an influx of leukocytes and epithelial layer damages. Fecal calprotectin, a leukocyte cytosolic protein, correlates with disease activity and is used as a marker of gut inflammation. Interestingly, patients with Alzheimer's disease have elevated stool level of calprotectin that is inversely associated with serum concentration of essential aromatic amino acids and reflects an abnormal gut barrier [6]. Insulin resistance may play a role in neurodegenerative diseases as well. Glucagon-like peptide one (GLP-1) is a potent insulin tropic hormone produced by L cells in the distal gut during the postprandial phase. Preclinical studies have indicated that liraglutide, a GLP-1 analogue, has anti-apoptotic, anti-inflammatory, antioxidant, and neuroprotective effects in stroke and Alzheimer's disease [7]. Patients that underwent colectomy have lower postprandial GLP-1 levels [8] due to the loss of L cells. Therefore, surgical interventions on gastrointestinal tract could alter the progression of neurodegenerative diseases.

Parkinson's disease (PD) is an idiopathic neurodegenerative disorder associated with destruction of the nigrostriatal pathway. Lewy bodies, a hallmark of PD, are predominantly composed of the presynaptic protein α-synuclein [9]. Interestingly, patients with de novo PD have abnormal colonic permeability, motility, and accumulation of α-synuclein in the enteric neurons [10]. According to Braak hypothesis [11], the enteric nervous system serves as a conduit for disease progression and in preclinical disease models; sympathetic and parasympathetic nerve interruption can halt disease progression. Gut barrier defects also lead to increased circulating levels

of gut microbiota-derived LPS and systemic inflammation. Systematic administration of LPS (a pro-inflammatory molecule) in mouse models of PD leads to progression of nigral pathology and, thus, brings further evidence into pathological gut-brain axis theory [10].

Multiple sclerosis (MS) is the prototypical inflammatory disease of the CNS. No specific cause has been identified, but both clinical and preclinical studies (experimental autoimmune encephalitis, EAE) indicate that Th1 and Th17 cells derived in the periphery play an important role [12]. Intriguingly, MS shares immune-related gene polymorphisms with inflammatory bowel diseases and type 1 diabetes. Not surprisingly, dysbiosis and gut barrier defects are present in all these conditions. Altered intestinal morphology and Th1/Th17 cell infiltration precede the development of EAE and are associated with overexpression of the tight junction regulator zonulin [13]. Furthermore, overexpression of zonulin is also seen in patients with celiac sprue (CS) exposed to dietary gluten [14]. Importantly, up to twenty percent of these patients may have demyelinating lesions in the CNS [15]. Furthermore, CS is more prevalent in MS patients and their first-degree relatives [16]. Similar to IBD, antibiotic treatment can ameliorate the severity of EAE and underscores the importance of gut commensal microorganisms in regulating the blood-brain barrier function and CNS response to inflammation.

Nutrient Effect on Gut-Brain Inflammatory Axis

The nutrient effect on central nervous system (CNS) function has been shown to be either direct via metabolites generated during digestion or indirect through complex interaction with gut microbiota and the gut-associated lymphatic tissue (GALT) (Fig. 8.1).

Dietary Metabolites: CNS Pathway

The blood-brain barrier assures selective transport of various dietary-derived compounds that circulate in the plasma. In general, the ability to diffuse across the barrier is a function of the lipid solubility, but molecules with low solubility will benefit from a carrier-mediated transport. Furthermore, vitamins and essential amino acids are not synthesized in the brain and require specific transport systems; their availability in the brain is dependent on the diet and could be limited by substrate competition [17].

Western diet has been associated with obesity epidemic and a rising incidence of autoimmune disorders [18]. Saturated fat has been linked to the development of MS [19] and restriction of animal fat may alter the disease course. The relative concentration of dietary polyunsaturated fatty acids (PUFA) can alter the outcome of chronic inflammation. N-3 fatty acids are found in plant oils (i.e., α-linolenic acid) and fish

oils (i.e., EPA and DHA) and exert anti-inflammatory activity. On the other hand, excess of n-6 fatty acids (i.e., linoleic acid) may promote an inflammatory milieu by stimulating the production of arachidonic acid (AA) and downstream production of pro-inflammatory prostaglandins, leukotrienes, and thromboxanes. N-3 fatty acid-derived resolvins D1 and E1 can decrease microglial production of TNFα and IL-6 and, thus, mitigate neuroinflammatory conditions [20]. In an animal model of cuprizone-induced demyelination, n-3 PUFAs docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids were able to reduce the neurodegenerative process and improve motor and cognitive functions [21]. Mechanistically, this was linked to decreased microglial production of interferon gamma (IFNγ), increased myelin phagocytosis, and a shift toward M2 phenotype. Similar protective activity was observed in vascular neurodegenerative disease models [22]. Several randomized control studies have addressed the role of n-3 PUFAs (EPA and DHA) in patients with multiple sclerosis [23, 24]. Until now, there was no significant effect on MRI disease activity, rate, or severity of relapses. All studies used comparable daily doses of EPA and DHA.

Molecular mimicry between host and microbial components may lead to pathological activation of the immune system. A study in children with central nervous system (CNS) inflammatory demyelination revealed autoreactive T cells, as well as abnormal T cell responses against multiple cow-milk proteins [25], such as butyrophilin (BTN). Interestingly, butyrophilin resembles the MS autoantigen, myelin oligodendrocyte glycoprotein (MOG). In a rat model of EAE, BTN administration resulted in accumulation of meningeal and perivascular infiltrates of T cells and macrophages throughout the brain [26]. Furthermore, MOG-specific T cells that were activated by exposure to BTN or/and transfer of BTN-induced T cells, reacted to MOG antigen, thus proving the molecular mimicry mechanism. Based on clinical and preclinical studies, MS patients may choose to refrain from consuming cow's milk, a rich source of BTN. On the other hand, this cross-reactivity with the MS-putative antigen may be exploited to induce immune tolerance and reduce disease activity [26] by modulating Th1, Th17, and Treg responses [27].

Prior exposure to viral antigen may generate T cell clones that cross react with food antigens. Patients with celiac sprue (CS) develop a chronic autoimmune response and gut barrier damage when exposed to dietary gluten peptides. It was proposed that adenovirus infection might promote the development of CS upon subsequent exposure to gluten [28]. Neurological complications such as cerebellar ataxia, gluten encephalopathy, multiple sclerosis, and peripheral neuropathies are not uncommon in CS [29]. Interestingly, influenza A and Epstein-Barr viral infections have been associated with higher relapse rate, and there is a known relationship between these events and the immune response to dietary antigens. Currently, the association between CS and MS remains controversial [30, 31], and a small observational study indicated no benefit of a gluten-free diet in MS patients [32].

It is known that the reactive oxygen species (ROS) promote inflammation and neuronal damage. Bioactive plant-derived molecules may act as antioxidants and ROS scavengers and thus exert a neuroprotective effect. Polyphenols present in vegetables, fruits, tea, spices, and wine have been shown to have protective effect in animal models of brain injury. In vivo and in vitro animal studies indicate that

polyphenols, such as flavonoids, can cross the blood-brain barrier [33, 34]. Flavonoids have been shown to modulate several pathways that regulate neuronal death (p38, JNK1/2) and survival (PI3K/Akt, ERK1/2, PKC) [35, 36]. In addition, they might regulate disease-specific processes. For example, plaque formation in Alzheimer's disease is largely due to accumulation of amyloid β and tau protein. The flavonoid myricetin can antagonize the aggregation of amyloid [37], while green and white tea extracts (epigallocatechin gallate) can function as acetylcholinesterase inhibitors, a cofactor of amyloid toxicity [38]. In a mouse model of Parkinson's disease, acacetin, a naturally occurring flavone, was able to protect dopaminergic neurons by blocking the production of nitric oxide, prostaglandin E2, and TNFα [39].

Food represent a mixture of compounds that may synergize or antagonize each other, and thus, activity of isolated phytochemicals components may not be readily translated in a disease modifying diet. For example, in a mouse model of ischemic stroke, the flavonols epicatechin and quercetin showed a protective effect on neuronal survival only when administered together [40].

Diet-Gut Microbiota: CNS Pathway

Dietary interventions that affect gut microbial species will alter gut barrier function and promote local and distant organ dysfunction. A study comparing children from Burkina Faso and an urban European area correlated a high ratio of gut *Bacteroidetes* to *Firmicutes* bacteria with higher dietary fiber intake [41].

Moreover, two of the bacterial species, *Prevotella* and *Xylanibacter* associated with enhanced production of short-chain fatty acids (SCFAs), were absent in the European cohort. Studies have shown that colon microbiota in humans consuming high-fiber diet will benefit from a higher production of short-chain fatty acids (SCFA) known to block inflammation and cancer development. On the other hand, diet-induced gut dysbiosis may promote neurodegenerative and neuroinflammatory processes. In a recent study, transfer of gut microbiota from high-fat diet fed mice to nonobese mice on regular chow resulted in abnormal cognitive and behavioral changes [42]. MS patients, similar to those with inflammatory bowel diseases or obesity, exhibit a decrease in *Clostridia* clusters XIVa and IV, which consist of bacterial species associated with the production of anti-inflammatory SCFA [43].

SCFA production is dependent on dietary intake of fiber and specific microbiota. Bacterial fermentation of dietary fiber produces acetate, propionate, and butyrate. Acetate is the most abundant SCFA in circulation followed by propionate and butyrate. SCFAs may cross the BBB [44] and modulate neuronal metabolic function, as well as gene transcription by their histone deacetylase inhibitory activity [45]. Recent studies have indicated an imbalance in the histone acetylation/deacetylation in patients with Parkinson's disease [46]. Excessive histone deacetylation may alter the expression of genes involved in neuronal survival. Thus, histone deacetylase (HDAC) inhibitors such as SCFAs may be beneficial in neurodegenerative condi-

tions. Furthermore, in vitro studies showed that butyrate protects cerebellar granule neurons, microglia, and astrocytes against LPS-induced secretion of pro-inflammatory cytokines [47]. The observed effect appeared to be mediated by epigenetic modulation of nuclear factor kappa B (NF-kB) binding to the promoter region of these cytokines and consistent with the HDAC inhibitory activity of SCFAs.

Mediterranean diet (MD) promotes consumptions of cereals, legumes, nuts, and fruits and has been shown to reduce the incidence of metabolic disorders and inflammatory and degenerative conditions. Alterations in gut microbiota by MD may underlie these beneficial effects. A 6-week study in patients with Crohn's disease showed that MD ameliorated inflammation and dysbiosis. Crohn's disease patients on Mediterranean diet had higher frequency of *Bacteroides* and *Clostridium* and reduced presence of *Proteobacteria* and *Bacillaceae*, similar to healthy controls [48]. Thus, diet-induced microbiome changes can modulate the outcome of chronic, autoimmune inflammation. Given the higher amount of polyphenols, n-3 PUFAs, and fiber in MD compared to Western diet, Mediterranean dietary interventions can effectively modulate gut microbiota and the gut barrier and change the outcome of various neurodegenerative illnesses.

Diet: GALT: CNS Pathway

Gut barrier and gut-associated lymphoid tissue (GALT) can modulate distant organ inflammation via dietary and microbial antigens, as well as generation of autoreactive T cells. Pro-inflammatory cytokines generated during states of intestinal inflammation may alter brain function. In a mouse model of dextran sodium sulfate (DSS) colitis, chronic intestinal inflammation reduced hippocampal neurogenesis [49]. Analysis of hippocampal tissue in mice that developed colitis revealed increased expression of pro-inflammatory cytokines TNF-α and IL-1. Furthermore, in vivo and in vitro experiments demonstrated that pro-inflammatory cytokines reduced hippocampal neurogenesis by upregulating the cell cycle "molecular brake" p21 and decreasing the levels of neuron proliferation markers doublecortin (DCX) and Ki-67 [50].

High-fat diet induces gut barrier defects and promotes naïve T-cell polarization into Th1 and Th17 phenotypes [51]. Peripherally generated T cells can cross the BBB and may alter the course of neuroinflammatory conditions such as MS, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. Antibiotic treatment ameliorates inflammation in animal models of inflammatory bowel diseases and experimental autoimmune encephalitis (EAE) [52]. Analysis of intestinal Peyer's patches (PP) and mesenteric lymph nodes (MLN) in a mouse model of EAE indicated that oral antibiotics enhanced the frequency of FoxP3 T_{reg} cells (anti-inflammatory) while decreasing the production of cytokines inductive of Th1 and Th17 pro-inflammatory cells [52]. The enhanced conversion of naïve T cells into FoxP3 T_{reg} cells was linked to increased number of tolerogenic $CD11c^{high}CD103^{+dendritic}$

cells in PP and MLN. Thus, immune cell polarization in the gut environment could alter the course of brain inflammatory diseases.

Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor with important homeostatic functions. Also, it plays an important role in pathological processes, such as xenobiotic metabolism, cancer, and immunity. In a DSS model of inflammatory bowel diseases, AhR knockout mice succumbed early in the process of colitis due to widespread barrier defects and uncontrolled inflammation [53]. On the other hand, $AhR^{-/+}$ heterozygote mice were protected from colitis, whereas $AhR^{+/+}$ wild mice developed severe inflammation. Mechanistically, we observed that the increased AhR expression in the wild-type mice was associated with increased markers of pro-inflammatory Th1 and Th17 cells and a significant decrease in the tolerogenic Treg cells [53]. It was previously shown that both exogenous and endogenous components activate the AhR pathway. Intriguingly, the fine line between homeostatic and pathological effects depends on the dose and the type of ligand. Dioxin-type xenobiotics, such as those present in cigarette smoke, promote chronic inflammation [54, 55]. On the other hand, dietary tryptophan and cruciferous vegetables are rich sources of AhR ligands that modulate the immune response and help establish immune tolerance, increasing resistance to pathogenic bacteria and fungi [56, 57]. Furthermore, several preclinical studies assessed the effect of dietary AhR ligands (baicalein, daidzein, resveratrol, naringenin, sulforaphane) on experimental autoimmune encephalitis. Sulforaphane, a compound obtained from cruciferous vegetables such as broccoli, Brussels sprouts, or cabbages, inhibited the development and severity of EAE [58]. Mechanistically, this compound had pleiotropic effects: maintenance of blood-brain barrier (claudin-5, occludin, MMP-9), anti-inflammatory (Th17, IL-10), and antioxidant (Nrf2/ARE pathway). Thus, plant-derived AhR ligands hold promise in the treatment of multiple sclerosis as well as neurodegenerative diseases.

Conclusion

A significant progress has been made in understanding the molecular mechanisms of neuroinflammatory and neurodegenerative diseases. Most of the efforts are geared toward drug delivery devices that enhance passage across the blood-brain barrier as well as drugs that target disease-specific pathways. On the other hand, understanding the role that diet plays in the immune cell activation, BBB integrity, and intracellular mechanism that promote clearance of neurotoxic host-derived proteins (α-synuclein, β-amyloid) can offer powerful complimentary interventions in MS and Parkinson's and Alzheimer's disease. Furthermore, it is crucial to understand the complex interactions between diet and gut microbiome. Diet-derived immunomodulatory and neuroprotective products depend on microbiome metabolism. Similarly, survival of health-promoting microbial communities requires specific dietary-derived prebiotics. Thus, although Mediterranean diet may be seen as

a more general nutritional intervention, designing diets based on patient-specific microbiome and metabolomic data can offer customized treatments and fulfill the tenets of personalized medicine.

References

1. Pachter JS, de Vries HE, Fabry Z. The blood–brain barrier and its role in immune privilege in the central nervous system. J Neuropathol Exp Neurol. 2003;62(6):593–604.
2. Ifergan I, et al. The blood–brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. Brain. 2008;131(Pt 3):785–99.
3. Walker DG, Lue LF. Immune phenotypes of microglia in human neurodegenerative disease: challenges to detecting microglial polarization in human brains. Alzheimers Res Ther. 2015;7(1):56.
4. Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol. 2011;11(11):775–87.
5. da Fonseca AC, et al. The impact of microglial activation on blood–brain barrier in brain diseases. Front Cell Neurosci. 2014;8:362.
6. Leblhuber F, et al. Elevated fecal calprotectin in patients with Alzheimer's dementia indicates leaky gut. J Neural Transm (Vienna). 2015;122(9):1319–22.
7. Candeias EM, et al. Gut-brain connection: the neuroprotective effects of the anti-diabetic drug liraglutide. World J Diabetes. 2015;6(6):807–27.
8. Robertson MD, et al. The influence of the colon on postprandial glucagon-like peptide 1 (7–36) amide concentration in man. J Endocrinol. 1999;161(1):25–31.
9. Bruck D, et al. Glia and alpha-synuclein in neurodegeneration: a complex interaction. Neurobiol Dis. 2016;85:262–74.
10. Kelly LP, et al. Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease. Mov Disord. 2014;29(8):999–1009.
11. Braak H, et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197–211.
12. Legroux L, Arbour N. Multiple sclerosis and T lymphocytes: an entangled story. J Neuroimmune Pharmacol. 2015;10(4):528–46.
13. Nouri M, et al. Intestinal barrier dysfunction develops at the onset of experimental autoimmune encephalomyelitis, and can be induced by adoptive transfer of auto-reactive T cells. PLoS One. 2014;9(9):e106335.
14. Drago S, et al. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006;41(4):408–19.
15. Kieslich M, et al. Brain white-matter lesions in celiac disease: a prospective study of 75 diet-treated patients. Pediatrics. 2001;108(2):E21.
16. Rodrigo L, et al. Prevalence of celiac disease in multiple sclerosis. BMC Neurol. 2011;11:31.
17. Gardiner RM. Transport of amino acids across the blood–brain barrier: implications for treatment of maternal phenylketonuria. J Inherit Metab Dis. 1990;13(4):627–33.
18. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132(6):2169–80.
19. Swank RL, Goodwin JW. How saturated fats may be a causative factor in multiple sclerosis and other diseases. Nutrition. 2003;19(5):478.
20. Rey C, et al. Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. Brain Behav Immun. 2015. pii: S0889-1591(15)30075-1. doi: 10.1016/j.bbi.2015.12.013.
21. Chen S, et al. n-3 PUFA supplementation benefits microglial responses to myelin pathology. Sci Rep. 2014;4:7458.
22. Berman DR, et al. Docosahexaenoic acid pretreatment confers neuroprotection in a rat model of perinatal cerebral hypoxia-ischemia. Am J Obstet Gynecol. 2009;200(3):305.e1–6.

23. Weinstock-Guttman B, et al. Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients. Prostaglandins Leukot Essent Fatty Acids. 2005; 73(5):397–404.
24. Torkildsen O, et al. omega-3 fatty acid treatment in multiple sclerosis (OFAMS Study): a randomized, double-blind, placebo-controlled trial. Arch Neurol. 2012;69(8):1044–51.
25. Banwell B, et al. Abnormal T-cell reactivities in childhood inflammatory demyelinating disease and type 1 diabetes. Ann Neurol. 2008;63(1):98–111.
26. Stefferl A, et al. Butyrophilin, a milk protein, modulates the encephalitogenic T cell response to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis. J Immunol. 2000;165(5):2859–65.
27. Mana P, et al. Tolerance induction by molecular mimicry: prevention and suppression of experimental autoimmune encephalomyelitis with the milk protein butyrophilin. Int Immunol. 2004;16(3):489–99.
28. Lahdeaho ML, et al. Antibodies to E1b protein-derived peptides of enteric adenovirus type 40 are associated with celiac disease and dermatitis herpetiformis. Clin Immunol Immunopathol. 1993;69(3):300–5.
29. Bushara KO. Neurologic presentation of celiac disease. Gastroenterology. 2005;128(4 Suppl 1):S92–7.
30. Shor DB, et al. Gluten sensitivity in multiple sclerosis: experimental myth or clinical truth? Ann N Y Acad Sci. 2009;1173:343–9.
31. Nicoletti A, et al. Frequency of celiac disease is not increased among multiple sclerosis patients. Mult Scler. 2008;14(5):698–700.
32. Hewson DC. Is there a role for gluten-free diets in multiple sclerosis? Hum Nutr Appl Nutr. 1984;38(6):417–20.
33. Youdim KA, et al. Interaction between flavonoids and the blood–brain barrier: in vitro studies. J Neurochem. 2003;85(1):180–92.
34. Peng HW, et al. Determination of naringenin and its glucuronide conjugate in rat plasma and brain tissue by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 1998;714(2):369–74.
35. Levites Y, et al. Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (−)-epigallocatechin 3-gallate neuroprotective action. J Biol Chem. 2002;277(34):30574–80.
36. Zhang Z, et al. Baicalein protects against 6-OHDA-induced neurotoxicity through activation of Keap1/Nrf2/HO-1 and involving PKC alpha and PI3K/AKT signaling pathways. J Agric Food Chem. 2012;60(33):8171–82.
37. Ono K, et al. Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. J Neurochem. 2003;87(1):172–81.
38. Okello EJ, Leylabi R, McDougall GJ. Inhibition of acetylcholinesterase by green and white tea and their simulated intestinal metabolites. Food Funct. 2012;3(6):651–61.
39. Kim HG, et al. Acacetin protects dopaminergic cells against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neuroinflammation in vitro and in vivo. Biol Pharm Bull. 2012;35(8):1287–94.
40. Nichols M, et al. Synergistic neuroprotection by epicatechin and quercetin: activation of convergent mitochondrial signaling pathways. Neuroscience. 2015;308:75–94.
41. De Filippo C, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107(33):14691–6.
42. Bruce-Keller AJ, et al. Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity. Biol Psychiatry. 2015;77(7):607–15.
43. Miyake S, et al. Dysbiosis in the Gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to clostridia XIVa and IV clusters. PLoS One. 2015;10(9):e0137429.

44. Frost G, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun. 2014;5:3611.
45. Shah P, et al. Short chain fatty acids induce TH gene expression via ERK-dependent phosphorylation of CREB protein. Brain Res. 2006;1107(1):13–23.
46. Harrison IF, Dexter DT. Epigenetic targeting of histone deacetylase: therapeutic potential in Parkinson's disease? Pharmacol Ther. 2013;140(1):34–52.
47. Huuskonen J, et al. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. Br J Pharmacol. 2004;141(5):874–80.
48. Marlow G, et al. Transcriptomics to study the effect of a Mediterranean-inspired diet on inflammation in Crohn's disease patients. Hum Genomics. 2013;7:24.
49. Spalding KL, et al. Dynamics of hippocampal neurogenesis in adult humans. Cell. 2013;153(6):1219–27.
50. Couillard-Despres S, et al. Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci. 2005;21(1):1–14.
51. Gruber L, et al. High fat diet accelerates pathogenesis of murine Crohn's disease-like ileitis independently of obesity. PLoS One. 2013;8(8):e71661.
52. Ochoa-Reparaz J, et al. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol. 2009;183(10):6041–50.
53. Arsenescu R, et al. Role of the xenobiotic receptor in inflammatory bowel disease. Inflamm Bowel Dis. 2011;17(5):1149–62.
54. Arzuaga X, et al. Induction of gene pattern changes associated with dysfunctional lipid metabolism induced by dietary fat and exposure to a persistent organic pollutant. Toxicol Lett. 2009;189(2):96–101.
55. Arsenescu V, et al. Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. Environ Health Perspect. 2008;116(6):761–8.
56. Zelante T, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013;39(2):372–85.
57. Li Y, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell. 2011;147(3):629–40.
58. Li B, et al. Sulforaphane ameliorates the development of experimental autoimmune encephalomyelitis by antagonizing oxidative stress and Th17-related inflammation in mice. Exp Neurol. 2013;250:239–49.

Chapter 9
Guillain–Barré Syndrome and *Campylobacter jejuni* Enteritis

Nortina Shahrizaila and Nobuhiro Yuki

Abstract Guillain–Barré syndrome is an immune-mediated neuropathy that accounts for one of the most common acute neuromuscular paralysis worldwide. Characteristic to its history is an antecedent illness and this includes *Campylobacter jejuni* enteritis. The pathogenesis of *C. jejuni*-related Guillain–Barré syndrome has been extensively studied, and there is good evidence to support molecular mimicry between self and microbial components as the mechanism of disease. Self-antigens in the form of gangliosides which are predominantly cell-surface glycolipids highly expressed in nervous tissue share similar characteristics as lipo-oligosaccharides of *C. jejuni* outer membrane. Molecular mimicry has been demonstrated between GM1 ganglioside and lipo-oligosaccharide of *C. jejuni* isolated from Guillain–Barré syndrome patients. This includes the establishment of disease models by sensitisation of rabbits with GM1 and *C. jejuni* lipo-oligosaccharide. This chapter discusses the current understanding of Guillain–Barré syndrome following *C. jejuni* enteritis.

Keywords Acute motor axonal neuropathy • Anti-ganglioside antibodies • *Campylobacter jejuni* enteritis • Guillain–Barré syndrome • Molecular mimicry

Introduction

Guillain–Barré syndrome (GBS) is an immune-mediated neuropathy and is the leading cause of flaccid paralysis in the post-polio era. The syndrome was named after two French neurologists who described the condition presenting in two soldiers [1]. In a typical case of GBS, patients present with a history suggestive of a

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progressive sensorimotor neuropathy. Cerebrospinal fluid (CSF) analysis reveals albumin-cytological dissociation and nerve conduction studies support a neuropathy. The global incidence of GBS ranges from 0.4 to 4.0 (median 1.3) cases per 100,000 people annually, occurring slightly more often in adolescents and young adults than in children [2, 3].

Several studies have demonstrated the presence of a preceding illness prior to the development of GBS. This antecedent illness is likely to play an important role in the pathophysiology of GBS. In particular, GBS patients with an antecedent *Campylobacter jejuni* infection go on to develop an axonal form of GBS. The mechanism underlying the relationship between *C. jejuni* and axonal GBS lies in the concept of molecular mimicry. The hypothesis of molecular mimicry postulates that the structural similarities between microbial antigens and certain host antigens lead to the autoantibodies or autoreactive T cells induced by the antecedent infections to destroy both the microbial and host targets. GBS is one of the few conditions in which this hypothesis has been proven to hold true.

GBS and Its Related Conditions

Since its first clinical description in 1916, there have been many reports of variable presentations of an acute immune-mediated polyneuritis similar to GBS. Historically, Guillain also recognised various forms of GBS which he reported in 1938 and proposed a clinical classification that took into account four presentations: the lower form, the spinal and midbrain form, the midbrain form and polyradiculoneuropathy with impaired mentation. Clinical diagnostic criteria for GBS were introduced in 1978 following an increase in incidence after the swine flu vaccination programme [4]. The criteria were later validated and modified accordingly [2]. These sets of criteria have since been utilised in most reported works of GBS. However, the criteria were developed to enable non-neurologists to recognise GBS; thus, they were intentionally restrictive, requiring the presence of universal limb areflexia or hyporeflexia.

The electrodiagnosis of GBS plays an important part in confirming the presence of a neuropathy as well as characterising the type of nerve involvement. The electrodiagnosis of GBS can be divided into two forms, acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN).

The more recent GBS criteria outlined by the Brighton Collaboration GBS working group in 2011 aimed to standardise the collection and assessment of information, particularly on vaccination-related GBS or Miller Fisher syndrome (MFS) patients. The criteria allowed for data comparability between different geographical locations that have health-care settings that differ by availability of and access to health care [5]. It is now recognised that with the identification of several new phenotypes in the past 30 years, the clinical presentations are best represented as a disease spectrum which includes the extent of disease involvement, ranging from mild to severe. A new set of diagnostic criteria has since been recommended which is less restrictive and more inclusive of the possible presentations of GBS [6].

The subtypes of GBS have also been demonstrated to vary amongst different geographical regions. For instance, AIDP is common in North America and Europe, whereas AMAN have been more commonly found in studies in China, Japan and Mexico [7, 8]. MFS has also been described more frequently in Asian countries [9].

Antecedent Illness in GBS

What is remarkable in the history of patients with GBS is that the neuropathy is commonly preceded in the previous month by an infective episode. A recent systematic review of GBS estimated that 40–70 % of all GBS cases are preceded by an acute infectious illness, of which 22–53 % are upper respiratory infections and 6–26 % are gastrointestinal infections [10]. Several studies have demonstrated this to be true in GBS cases when compared to controls, including other neurological controls.

In 1964, a case–control study reported a history of respiratory infection in 48 % of GBS patients compared to only 18 % of controls [11]. In a retrospective review that compared GBS patients with patients presenting with Bell's palsy, a history of preceding infection was noted in 28/37 patients, 12 of whom had primarily gastrointestinal symptoms [12]. This was later followed by another study that looked prospectively at the association of antecedent illness and GBS, and the investigators found a relative risk of 4.1 for antecedent respiratory illness in GBS patients, whereas this risk was higher at 7.5 for gastrointestinal infections [13]. GBS patients would typically report respiratory infections occurring 1–2 weeks before neuropathy onset, whereas gastrointestinal infections would typically precede neuropathy by only 1 week.

C. jejuni is one of the most common causes of bacterial gastroenteritis, affecting almost 50 per 100,000 individuals in developed countries [14]. The first case of *C. jejuni* infection in GBS patients was reported in 1982 [15]. The authors described a 45-year-old man who developed GBS 2 weeks after *C. jejuni* enteritis. The enteritis was initially treated with erythromycin before the onset of symmetrical limb weakness. It is often difficult to isolate *C. jejuni* from stool samples at the time of GBS presentation because the bacteria are usually eliminated from the body within 16 days of infection [16] and before the onset of neurological symptoms, which normally begin 10 days to 3 weeks after the onset of diarrhoea. However, later studies were able to look at serological reaction to *C. jejuni* to detect a recent infection. Since then, several case–control studies have reported a significant association between antecedent *C. jejuni* infection and GBS.

A case–control study of 56 Australian GBS patients and 57 controls found evidence of a recent *C. jejuni* infection in 38 % of GBS patients [17]. The authors also found that in comparison to sera from patients with uncomplicated *C. jejuni* enteritis, the degree of IgG rise was significantly higher in GBS patients compared to IgA or IgM. The possibility of an immune-mediated cross-reaction between neural tissue and *C. jejuni* was considered. A separate study investigating a cohort of British GBS patients also found a higher percentage of patients who were seropositive for

C. jejuni infection compared to controls [13]. The risk of developing GBS was highest within the first 2 weeks of having had an infection, and *C. jejuni* infection was associated with a worse outcome in their cohort.

Two prospective case–control studies came later which provided convincing evidence that the association of *C. jejuni* with GBS was real [18, 19]. Rees and colleagues investigated 96 GBS and 7 MFS patients presenting over a period of 2 years and found significantly higher frequency of GBS patients with antecedent *C. jejuni* infection (26 % vs 2 %) [18]. The authors also found that a preceding *C. jejuni* infection was associated with axonal degeneration, slow recovery and severe residual disability. Similar studies were performed by the Dutch GBS group who investigated 154 GBS patients and found antecedent *C. jejuni* infection in 32 % followed by cytomegalovirus (13 %) and Epstein–Barr virus (10 %) [19]. The authors also found antecedent *C. jejuni* infection to be associated with axonal form as well as a more severe form of GBS. The exclusive association of antecedent *C. jejuni* and the axonal form of GBS has been questioned. The current electrodiagnostic criteria have limitations as they fail to recognise early reversible "demyelinating" features that are seen in acute motor conduction block neuropathy [20]. In a recent study of *C. jejuni*-related GBS, serial neurophysiology found that early demyelination reversed within 3 weeks of disease onset to reveal an axonal subtype of GBS suggesting the target antigens in *C. jejuni*-related GBS are likely to be axonal [21].

What has remained unresolved is the reason why, despite the common occurrence of *C. jejuni* infection worldwide, the risk of developing GBS remains very low. In the United States, for instance, the incidence of GBS was estimated to be 1 in every 1000 *C. jejuni* infections [22]. The Penner O serotyping has been used to characterise *C. jejuni* strains isolated from patients with GBS or MFS, and associations have been described in O:1, O:2, O:4, O:4/50, O:5, O:10, O:16, O:19, O:23, O:37, O:41, O:44 and O:64 [23]. However, later studies demonstrated wide variations in serotyping that failed to differentiate between *C. jejuni* associated with GBS and those without neurological complications [24]. These results suggest that specific Penner O serotyping is unlikely to be exclusively associated with GBS. Instead, sialylation of *C. jejuni* lipo-oligosaccharide (LOS) was more likely to play an important role in GBS pathogenesis. Ultimately, multiple factors are related to the microorganism as well as host factors that contribute towards the susceptibility of developing *C. jejuni*-related GBS.

The Pathophysiology of *C. jejuni*-Related GBS

The Role of Anti-ganglioside Antibodies

Gangliosides are a large family of glycosphingolipids, predominantly distributed on the cell-surface membrane and anchored in the external leaflet of the lipid bilayer by a ceramide moiety. The highly variable sialylated oligosaccharides are exposed

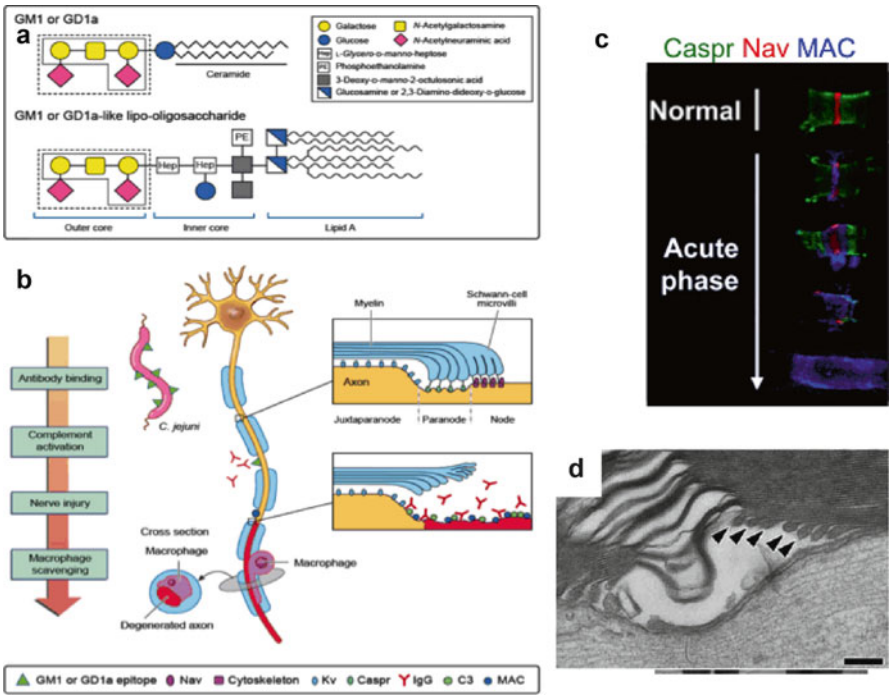

Fig. 9.1 Immunopathogenesis of *Campylobacter jejuni*-related Guillain–Barré syndrome. Panel (**a**) depicts the similarities between GM1 and *C. jejuni* lipo-oligosaccharide (LOS). Infection by *C. jejuni* bearing the ganglioside-like LOS (**b**) results in the production of anti-GM1 or anti-GD1a antibodies. The autoantibodies bind to the nodes of Ranvier in the spinal anterior roots. This results in complement activation, and membrane attack complex (MAC) is formed at the nodal axolemma. Immunofluoresence analyses of rabbit ventral roots (**c**) demonstrate how MAC formation result in the disappearance of voltage-gated sodium channel clusters at the nodes of Ranvier in the acute phase. Further evidence is seen on electron microscopy (**d**) of lengthening of the nodes of the rabbit ventral roots. These pathological changes go on to produce motor nerve conduction failure and muscle weakness

extracellularly [25] (Fig. 9.1). They are involved in maintaining the cell membrane structure and are likely to also be involved in cell growth, cell differentiation and cell to cell recognition. In GBS, antibodies against multiple different gangliosides have been reported, implicating these antibodies in the underlying disease pathogenesis [26].

The suggestion that anti-ganglioside antibodies may play an important role in the pathogenesis of GBS came following the report of a patient with a motor neuron disease-like disorder who had anti-GM1 IgM antibodies [27]. It was likely that the patient had multifocal motor neuropathy, although nerve conduction studies were not described. Anti-ganglioside antibodies were first reported in 1988 in five of 26 GBS patients [28]. The authors described the IgG titres as high in the acute phase of illness

and subsequently reduced as the clinical condition improved. In 1990, two patients with GBS following a bout of *C. jejuni* enteritis were found to have high titres of IgG antibodies against GM1. The patients also had, on neurophysiology, the AMAN subtype of GBS [29]. Other studies also demonstrated similar findings. Walsh and colleagues detected the predominant presence of IgG rather than IgM in 15 % of their GBS patients [30]. This group of patients also had a recent history of *C. jejuni* infection. In their study, Rees et al. demonstrated that significantly more *C. jejuni*-related GBS patients had anti-GM1 IgG antibodies, and this group of seropositive patients was also more likely to have axonal degeneration and less sensory disturbance than patients who were anti-GM1 antibody-negative patients. These findings were mirrored by those of Jacobs et al. who also reported a subgroup of GBS patients with anti-GM1 antibodies, who often had a more severe neuropathy in which there was predominantly distal distribution of weakness without sensory disturbances [31]. Apart from anti-GM1 antibodies, *C. jejuni*-related GBS have also been associated with antibodies to GM1b, GD1a and GalNAc-GD1a [32–34]. In the case of MFS, associations have been made with IgG antibodies against GQ1b and GT1a in patients who present with antecedent *C. jejuni* infection.

There may be situations where antibodies target pairs of gangliosides rather than a single ganglioside epitope in the pathogenesis of GBS. These are known as the antibodies against ganglioside complexes (GSC) [35]. In one study, 17 % had anti-GSC IgG antibodies, and they presented with a higher frequency of antecedent gastrointestinal infection and lower cranial nerve deficits [36]. Our own observations have demonstrated that GM1-like and GD1a-like LOSs form a GM1b epitope, inducing the development of anti-GM1b antibodies in GBS after *C. jejuni* enteritis. Mass spectrometry analysis confirmed that two isolates from GBS patients with anti-GM1b antibodies, but without anti-GM1 nor anti-GD1a antibodies, expressed both GM1/GD1a-like LOSs, but not concomitant GM1b-like LOS suggesting that complex of different bacterial structures form a new molecular mimicry [37]. The studies described provide evidence that IgG antibodies against gangliosides and GSC are associated with *C. jejuni* infection.

C. jejuni *and LOSs*

The development of GBS following an infective *C. jejuni* episode is linked to the cross-reactivity between antibodies that recognise both microbial and neural components to be similar. Specifically, the oligosaccharide core of LOS molecules expressed by *C. jejuni* is structurally similar to oligosaccharide moiety of the gangliosides.

In 1993, the authors demonstrated the LOS extracted from the *C. jejuni* isolated from a patient with GBS who had anti-GM1 IgG antibody reacted with cholera toxin (a specific ligand for the GM1-oligosaccharide) and could be purified by column chromatography. Gas–liquid chromatography–mass spectrometric analysis showed that the purified LOS has galactose (Gal), *N*-acetylgalactosamine (GalNAc)

and *N*-acetylneuraminic acid (NeuAc)—i.e. the sugar components of the GM1 ganglioside. ^{1}H nuclear magnetic resonance showed that the oligosaccharide structure (Galβ1–3 GalNAc β1–4[NeuAc α2–3] Galβ1) protrudes from the LOS core [38]. This terminal structure was identical to that of the terminal tetrasaccharide of the GM1 ganglioside. Similar findings were demonstrated in subsequent studies not only of GM1-like LOS but also GD1a-like, GD3-like and GT1a-like LOSs [39, 40].

In the case of anti-GSC antibodies, four patients with anti-GSC antibodies were analysed and found to cross-react with LOS from autologous *C. jejuni* isolates, suggesting that these antibodies were induced by *C. jejuni* LOS [35]. These findings set the stage for supporting the hypothesis that molecular mimicry was key to the pathogenesis of GBS, at least in *C. jejuni*-related GBS.

Molecular Mimicry and Animal Model

The concept of molecular mimicry postulates that the structural similarities between microbial antigens and certain host antigens lead to the production of autoantibodies or autoreactive T cells induced by antecedent infections to destroy both the microbial and host targets [41]. To conclude that a disease is triggered by molecular mimicry, four criteria should be satisfied as follows:

- Establishing an epidemiological association between the infectious agent and the immune-mediated disease
- Identifying T cells or antibodies directed against host target antigens in patients
- Identifying a microbial mimic of the target antigen
- Reproducing the disease in an animal model

In *C. jejuni*-related GBS, the first three criteria have been fulfilled as previously discussed. Robust epidemiological studies have established an association between antecedent *C. jejuni* infection with the development of GBS [13, 18]. The presence of anti-ganglioside antibodies and their structural similarities to the LOS of the *C. jejuni* isolates from GBS patients fulfil the second and third criteria. In order to fulfil the fourth criteria, GBS has to be reproduced in an animal model.

Efforts to reproduce disease in a mice model were attempted [42], but failed to produce the characteristic weakness seen in GBS. Instead, *C. jejuni* LOS inoculated into mice produced IgM against GM1 ganglioside. A further study immunised rabbits with GM1-like *C. jejuni* LOS from GBS-associated strains which produced high titres of IgG antibodies against GM1 but failed to produce the clinical signs in the rabbits [43]. The Japanese group, sensitising Japanese white rabbits with GM1 ganglioside, did subsequent studies. These rabbits developed acute flaccid paralysis and produced anti-GM1 antibodies. Pathological findings in their peripheral nerves showed predominant Wallerian-like degeneration with neither lymphocytic infiltration nor demyelination features. IgG antibodies were deposited on the axons of the anterior roots, internodal axolemmas and nodes of Ranvier. Cauda equina and spinal nerve root specimens from the paralysed rabbits showed macrophage infiltration in

the periaxonal space [44]. Surrounding myelin sheaths were almost intact. These findings correspond well with pathological findings for human AMAN [45, 46], confirming that GM1 was involved in the pathogenesis of the axonal form of GBS, AMAN.

In later studies, the rabbits were immunised with *C. jejuni* LOS bearing a GM1-like structure [47]. On sensitisation, the rabbits also developed anti-GM1 IgG antibodies and subsequent flaccid limb weakness. Autopsy studies demonstrated that their nerve roots had occasional macrophages in the periaxonal spaces surrounded by an almost intact myelin sheath. Axons of these nerve fibres showed various degrees of degeneration. Demyelination and remyelination features were rare. These findings again were compatible with the features of human AMAN. A successful animal model for AMAN subtype of GBS was developed, fulfilling the final criterion on the molecular mimicry. The AMAN animal model represents the first replica of human autoimmune disease in an animal model immunised by a microbial mimic of a self-antigen.

The Relationship Between Sialylated LOS and Different Clinical Presentations of GBS

In *C. jejuni*-associated GBS, the clinical presentation can be AMAN where patients present with flaccid paralysis or, in other cases, patients present with ataxia, ophthalmoplegia and areflexia which is characteristic of MFS.

The heterogeneity seen in the clinical presentations can be explained by the polymorphism of the gene encoding the enzyme, *Campylobacter* sialyltransferase (Cst-II), cst-II. *C. jejuni* strains from GBS patients expresses Cst-II which is involved in the sialylation of LOS [48]. Cst-II consists of 291 amino acids, and the 51st amino acid determines its enzymatic activity. Cst-II (Thr51) has only α2–3 sialyltransferase activity and produces GM1-like or GD1a-like LOS, whereas Cst-II (Asn51) has both α2–3 and α2–8 sialyltransferase activity and produces GT1a-like or GD1c-like LOS. *C. jejuni* isolates were collected from 105 patients with GBS (including variants) and from 65 patients with uncomplicated enteritis [48]. Patients infected with *C jejuni* cst-II (Thr51) were more frequently positive for anti-GM1 (88 % vs 35 %) and anti-GD1a IgG antibodies (52 % vs 24 %) and had limb weakness (98 % vs 71 %), whereas those with *C. jejuni* cst-II (Asn51) were more often positive for anti-GQ1b IgG antibodies (56 % vs 8 %) and had ophthalmoparesis (64 % vs 13 %) and ataxia (42 % vs 11 %).

The molecular pathogenesis of GBS or MFS subsequent to *C. jejuni* enteritis can be summarised as follows (Fig. 9.2): *C. jejuni* that carries cst-II (Thr51) can express GM1-like or GD1a-like LOS on its cell surface. Infection by such a *C. jejuni* strain may induce anti-GM1 or anti-GD1a IgG antibodies in some patients. The autoantibodies bind to GM1 or GD1a expressed on motor nerves, inducing AMAN. In contrast, *C. jejuni* carrying cst-II (Asn51) expresses GT1a-like or GD1c-like LOS on

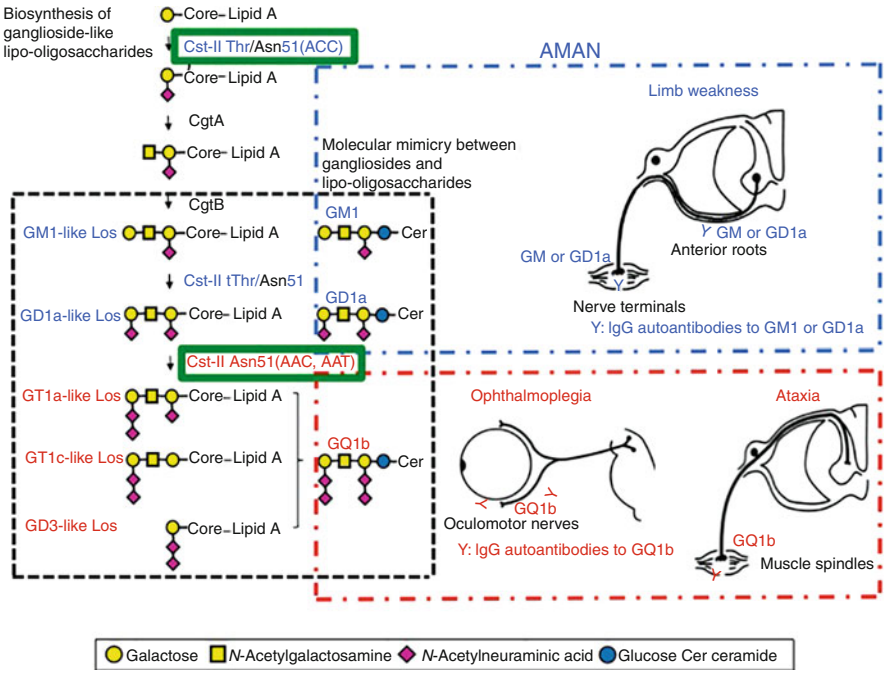

Fig. 9.2 Molecular mimicry between human gangliosides and *Campylobacter jejuni* lipo-oligosaccharides as a cause of Guillain–Barré syndrome and Miller Fisher syndrome (MFS). Panel (**a**) depicts the biosynthesis of *C. jejuni* lipo-oligosaccharide (LOS). Depending on the genetic polymorphism of Cst-II, different clinical patterns are seen. Cst-II (Thr51) strains lead to GM1-like and GD1a-like LOS which can induce the production of anti-GM1 or anti-GD1a antibodies. As GM1 and GD1a are strongly expressed in the peripheral motor nerves (**b**), anti-GM1 and anti-GD1a antibody-binding leads to limb weakness resulting in AMAN. Cst-II (Asn51) strains results in GT1a-like and GD1c-like LOS which can induce the production of anti-GQ1b antibodies. GQ1b is highly expressed in the oculomotor nerves and muscle spindles. (**c**) Following autoantibody binding, patients develop ophthalmoplegia and ataxia as is seen in MFS

its cell surface, and infection by such a strain may induce anti-GQ1b IgG antibodies in some patients. These autoantibodies bind to GQ1b that is expressed at the neuromuscular junctions of oculomotor muscles and on muscle spindles, inducing MFS.

Conclusion

In comparison to other antecedent infections, much progress has been made in our understanding of the pathogenesis of *C. jejuni*-associated GBS. This has as yet not been translated to more effective treatment options in GBS, where the mainstay of treatment is intravenous immunoglobulin or plasmapheresis. Current trials of complement inhibitors are underway. Future research is also likely to consider the

mechanisms by which susceptible individuals mount an autoimmune response following exposure to microorganisms.

References

1. Guillain G, Barré J, Strohl A. Sur un syndrome de radiculonévrite avec hyperalbuminose du liquide céphalo-rachidien sans réaction cellulaire. Remarques sur les caractères cliniques et graphiques des réflexes tendineux. Bulletins et mémoires de la Société des Médecins des Hôpitaux de Paris. 1916;40:1462–70.
2. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. Ann Neurol. 1990;27(Suppl):S21–4.
3. Hadden RDM, Cornblath DR, Hughes RAC, Zielasek J, Hartung HP, Toyka KV, et al. Electrophysiological classification of Guillain-Barré syndrome: clinical associations and outcome. Ann Neurol. 1998;44(5):780–8.
4. Asbury AK. Diagnostic considerations in Guillain-Barré syndrome. Ann Neurol. 1981; 9(Suppl):1–5.
5. Sejvar JJ, Kohl KS, Gidudu J, Amato A, Bakshi N, Baxter R, et al. Guillain-Barré syndrome and Fisher syndrome: case definitions and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2011;29(3):599–612.
6. Wakerley BR, Uncini A, Yuki N, GBS Classification Group. Guillain-Barré and Miller Fisher syndromes: new diagnostic classification. Nat Rev Neurol. 2014;10(9):537–44.
7. Paradiso G, Tripoli J, Galicchio S, Fejerman N. Epidemiological, clinical, and electrodiagnostic findings in childhood Guillain-Barré syndrome: a reappraisal. Ann Neurol. 1999;46(5):701–7.
8. Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M, Yuki N. Axonal Guillain-Barré syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. Ann Neurol. 2000;48(4):624–31.
9. Shahrizaila N, Goh KJ, Kokubun N, Tan AH, Tan CY, Yuki N. Sensory nerves are frequently involved in the spectrum of Fisher syndrome. Muscle Nerve. 2014;49(4):558–63.
10. McGrogan A, Madle GC, Seaman HE, de Vries CS. The epidemiology of Guillain-Barré syndrome worldwide: a systematic literature review. Neuroepidemiology. 2009;32(2):150–63.
11. Melnick SC, Flewett TH. Role of Infection in the Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry. 1964;27:395–407.
12. Kennedy RH, Danielson MA, Mulder DW, Kurland LT. Guillain-Barré syndrome: a 42-year epidemiologic and clinical study. Mayo Clin Proc. 1978;53(2):93–9.
13. Winer JB, Hughes RAC, Anderson MJ, Jones DM, Kangro H, Watkins RP. A prospective study of acute idiopathic neuropathy: II. antecedent events. J Neurol Neurosurg Psychiatry. 1988;51(5):613–8.
14. Janssen R, Krogfelt KA, Cawthraw SA, van Pelt W, Wagenaar JA, Owen RJ. Host-pathogen interactions in *Campylobacter* infections: the host perspective. Clin Microbiol Rev. 2008;21(3):505–18.
15. Rhodes KM, Tattersfield AE. Guillain-Barré syndrome associated with *Campylobacter* infection. Br Med J (Clin Res Ed). 1982;285(6336):173–4.
16. Svedhem A, Kaijser B. *Campylobacter fetus subspecies jejuni*: a common cause of diarrhea in Sweden. J Infect Dis. 1980;142(3):353–9.
17. Kaldor J, Speed BR. Guillain-Barré syndrome and *Campylobacter jejuni*: a serological study. Br Med J (Clin Res Ed). 1984;288(6434):1867–70.
18. Rees JH, Soudain SE, Gregson NA, Hughes RAC. *Campylobacter jejuni* infection and Guillain-Barré syndrome. N Engl J Med. 1995;333(21):1374–9.
19. Jacobs BC, Rothbarth PH, van der Meché FGA, Herbrink P, Schmitz PIM, de Klerk MA, et al. The spectrum of antecedent infections in Guillain-Barré syndrome: a case–control study. Neurology. 1998;51(4):1110–5.

20. Uncini A, Kuwabara S. Electrodiagnostic criteria for Guillain-Barré syndrome: a critical revision and the need for an update. Clin Neurophysiol. 2012;123(8):1487–95.
21. Kokubun N, Shahrizaila N, Koga M, Hirata K, Yuki N. The demyelination neurophysiological criteria can be misleading in *Campylobacter jejuni*-related Guillain-Barré syndrome. Clin Neurophysiol. 2013;124(8):1671–9.
22. Mishu B, Blaser MJ. Role of infection due to *Campylobacter jejuni* in the initiation of Guillain-Barré syndrome. Clin Infect Dis. 1993;17(1):104–8.
23. Nachamkin I, Allos BM, Ho T. *Campylobacter* species and Guillain-Barré syndrome. Clin Microbiol Rev. 1998;11(3):555–67.
24. Endtz HP, Ang CW, van den Braak N, Duim B, Rigter A, Price LJ, et al. Molecular characterization of *Campylobacter jejuni* from patients with Guillain-Barré and Miller Fisher syndromes. J Clin Microbiol. 2000;38(6):2297–301.
25. Ledeen RW, Yu RK. Gangliosides: structure, isolation, and analysis. Methods Enzymol. 1982;83:139–91.
26. Shahrizaila N, Yuki N. Antiganglioside antibodies in Guillain-Barré syndrome and its related conditions. Expert Rev Neurother. 2011;11(9):1305–13.
27. Freddo L, Yu RK, Latov N, Donofrio PD, Hays AP, Greenberg HS, et al. Gangliosides GM1 and GD1b are antigens for IgM M-protein in a patient with motor neuron disease. Neurology. 1986;36(4):454–8.
28. Ilyas AA, Willison HJ, Quarles RH, Jungalwala FB, Cornblath DR, Trapp BD, et al. Serum antibodies to gangliosides in Guillain-Barré syndrome. Ann Neurol. 1988;23(5):440–7.
29. Yuki N, Yoshino H, Sato S, Miyatake T. Acute axonal polyneuropathy associated with anti-GM1 antibodies following *Campylobacter* enteritis. Neurology. 1990;40(12):1900–2.
30. Walsh FS, Cronin M, Koblar S, Doherty P, Winer J, Leon A, et al. Association between glycoconjugate antibodies and *Campylobacter* infection in patients with Guillain-Barré syndrome. J Neuroimmunol. 1991;34(1):43–51.
31. Jacobs BC, van Doorn PA, Schmitz PIM, Tio-Gillen AP, Herbrink P, Visser LH, et al. *Campylobacter jejuni* infections and anti-GM1 antibodies in Guillain-Barré syndrome. Ann Neurol. 1996;40(2):181–7.
32. Kusunoki S, Chiba A, Kon K, Ando S, Arisawa K, Tate A, et al. *N*-Acetylgalactosaminyl GD1a is a target molecule for serum antibody in Guillain-Barré syndrome. Ann Neurol. 1994;35(5):570–6.
33. Kusunoki S, Iwamori M, Chiba A, Hitoshi S, Arita M, Kanazawa I. GM1b is a new member of antigen for serum antibody in Guillain-Barré syndrome. Neurology. 1996;47(1):237–42.
34. Ho TW, Willison HJ, Nachamkin I, Li CY, Veitch J, Ung H, et al. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barré syndrome. Ann Neurol. 1999;45(2):168–73.
35. Kaida K, Morita D, Kanzaki M, Kamakura K, Motoyoshi K, Hirakawa M, et al. Ganglioside complexes as new target antigens in Guillain-Barré syndrome. Ann Neurol. 2004;56 (4):567–71.
36. Kaida K, Morita D, Kanzaki M, Kamakura K, Motoyoshi K, Hirakawa M, et al. Antiganglioside complex antibodies associated with severe disability in GBS. J Neuroimmunol. 2007;182(1–2):212–8.
37. Koga M, Gilbert M, Li J, Yuki N. Complex of GM1- and GD1a-like lipo-oligosaccharide mimics GM1b, inducing anti-GM1b antibodies. PLoS One. 2015;10(4):e0124004.
38. Yuki N, Taki T, Inagaki F, Kasama T, Takahashi M, Saito K, et al. A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. J Exp Med. 1993;178(5):1771–5.
39. Aspinall GO, Fujimoto S, McDonald AG, Pang H, Kurjanczyk LA, Penner JL. Lipopolysaccharides from *Campylobacter jejuni* associated with Guillain-Barré syndrome patients mimic human gangliosides in structure. Infect Immun. 1994;62(5):2122–5.
40. Prendergast MM, Lastovica AJ, Moran AP. Lipopolysaccharides from *Campylobacter jejuni* O:41 strains associated with Guillain-Barré syndrome exhibit mimicry of GM1 ganglioside. Infect Immun. 1998;66(8):3649–55.

41. Witebsky E, Rose NR, Terplan K, Paine JR, Egan RW. Chronic thyroiditis and autoimmunization. J Am Med Assoc. 1957;164(13):1439–47.
42. Wirguin I, Briani C, Suturkova-Milosevic L, Fisher T, Della-Latta P, Chalif P, et al. Induction of anti-GM1 ganglioside antibodies by *Campylobacter jejuni* lipopolysaccharides. J Neuroimmunol. 1997;78(1–2):138–42.
43. Ang CW, Endtz HP, Jacobs BC, Laman JD, de Klerk MA, van der Meché FGA, et al. *Campylobacter jejuni* lipopolysaccharides from Guillain-Barré syndrome patients induce IgG anti-GM1 antibodies in rabbits. J Neuroimmunol. 2000;104(2):133–8.
44. Susuki K, Rasband MN, Tohyama K, Koibuchi K, Okamoto S, Funakoshi K, et al. Anti-GM1 antibodies cause complement-mediated disruption of sodium channel clusters in peripheral motor nerve fibers. J Neurosci. 2007;27(15):3956–67.
45. Hafer-Macko C, Hsieh S-T, Li CY, Ho TW, Sheikh K, Cornblath DR, et al. Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. Ann Neurol. 1996;40(4):635–44.
46. McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, et al. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol. 1993;33(4):333–42.
47. Yuki N, Susuki K, Koga M, Nishimoto Y, Odaka M, Hirata K, et al. Carbohydrate mimicry between human ganglioside GM1 and *Campylobacter jejuni* lipooligosaccharide causes Guillain-Barré syndrome. Proc Natl Acad Sci U S A. 2004;101(31):11404–9.
48. Koga M, Takahashi M, Masuda M, Hirata K, Yuki N. *Campylobacter* gene polymorphism as a determinant of clinical features of Guillain-Barré syndrome. Neurology. 2005;65(9):1376–81.

Chapter 10
Gut Microbiota: A Possible Role in the Pathogenesis of Multiple Sclerosis

Takashi Yamamura

Abstract Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system, in which both genetic and environmental factors are involved. Prevalence of MS is increasing remarkably in Asian countries including Japan, indicating a role of environmental factors related to westernization of lifestyle. Recent studies in immunology have demonstrated the dependency of pathogenic or regulatory lymphocytes on the gut microbiota component. Based on the epidemiological data in human and mouse immunology studies, we have been hypothesizing that alterations in the gut microbiota may underlie the pathogenesis of MS at least in Japan. Very recently, analysis of the bacterial 16S ribosomal RNA (rRNA) gene by using a high-throughput culture-independent pyrosequencing method provided evidence of a moderate dysbiosis in the structure of gut microbiota in Japanese patients with MS. Furthermore, we have identified 21 species that showed significant differences in relative abundance in MS as compared with healthy subjects, 2 increased and 19 reduced. The taxa reduced in MS comprised primarily of clostridial species belonging to *Clostridia* clusters XIVa and IV. Correcting the dysbiosis and altered gut microbiota might deserve consideration as a potential strategy for the prevention and treatment of MS.

Keywords Multiple sclerosis • Gut microbiota • Dysbiosis • Autoimmune disease • Clostridium • Regulatory T cells • *Faecalibacterium prausnitzii*

Introduction

Human gut is inhabited and colonized by trillions of commensal bacteria, fungi, and viruses, which are collectively referred to as the gut microbiota. Recent studies have demonstrated that gut microbiota interacts with the host immune system and plays an essential role in keeping the health and preventing disease conditions [1]. Previous works in the field of gastroenterology showed that compositions of fecal microbiota

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are significantly biased in inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease [2, 3]. Parallel research in rodent demonstrated that intestinal bacteria are involved in the pathogenesis of IBD models [4, 5]. More recently, it has been revealed that an alteration in the gut commensal flora is not only associated with IBD, but also with obesity, diabetes, cancer, and autoimmune diseases like multiple sclerosis (MS) [6, 7]. Curiously, patients with these diseases are increasing in developed countries, including Japan, where "westernized" lifestyle, higher intake of high-fat, low-fiber food, or early exposure to antibiotics is prevailing over last decades [8]. Nowadays, dysbiosis of human gut microbiota can be demonstrated by comprehensive genome analysis for bacterial 16S sequences or metagenome analysis. In this short review article, current understanding on the role of gut microbiota in MS is overviewed.

What Is the Relationship Between MS and Inflammatory Bowel Disease?

MS is a chronic autoimmune disease of the central nervous system (CNS), typically characterized by recurrent episodes of neurological disabilities and presence of multiple demyelinating foci in the CNS. MS is continuously increasing in developed countries over last decades [8, 9]. Although MS used to be a very rare disease in Japan, we are witnessing a very remarkable increase of patients with MS in Japan (Fig. 10.1a). This increase could not be explained by better awareness of the disease or advancement in diagnostic skills or facilities [10], and therefore nongenetic, environmental factors should be considered. Known environmental risk factors for MS include lower exposure to sunlight resulting in vitamin D3 deficiency, EB virus infection, and cigarette smoking [11]. There is no evidence that Japanese are exposed more significantly to these risk factors during last decades. High salt intake is also proposed to be a risk factor for MS [12]. However, salt intake is actually decreasing in Japan.

At the turn of centuries, the role of commensal bacteria in shaping lymphocyte repertoire started to attract the attention of immunologists [1], opening the gate to the research into microbiota-immune interactions. We are aware that Crohn's disease and ulcerative colitis are also increasing in Japan in parallel with MS (Fig. 10.1b). Is it reasonable to hypothesize that MS and IBD may share common pathways leading to occurrence of the inflammatory destruction? Although even nonexperts accepted the possible pathogenic role of altered gut environments in IBD, it took some more years till people started to consider if a brain disease MS could be associated with altered gut flora.

Roles of Gut Commensal Flora in Animal Models of MS

It remained obscure for years whether the gut microbiota could affect systemic immune responses beyond the gut. To answer the question, an animal model of MS, experimental autoimmune encephalomyelitis (EAE), has significantly contributed

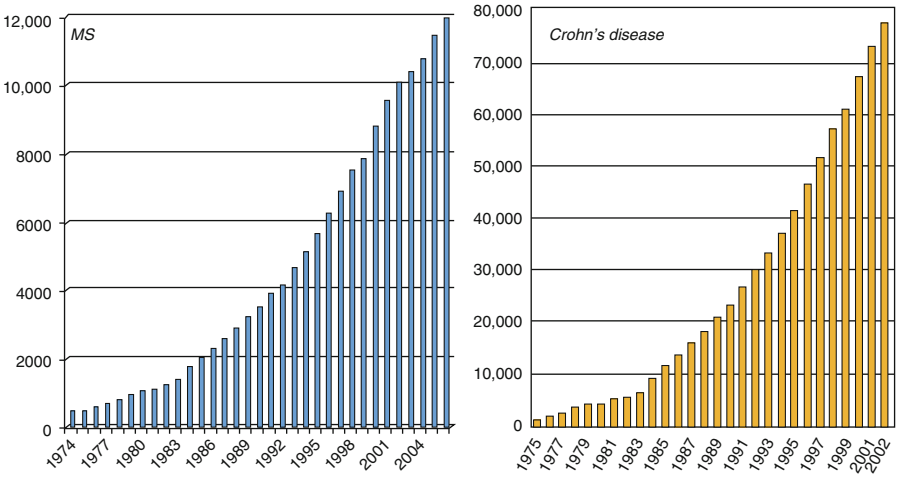

Fig. 10.1 Increase of multiple sclerosis (*MS*) and Crohn's disease in Japan

to our understanding. In 2008, we investigated if antibiotic treatment altering gut flora compositions might affect development of EAE. As reported by Yokote et al. [13], we induced EAE with MOG 35–55 peptide in B6 mice. Before and during induction phase, drinking water, containing nonabsorbing antibiotics kanamycin, colistin, and vancomycin, was given to the mice. The antibiotics treatment significantly altered the composition of intestinal microbiota but also reduced the clinical and pathological signs of EAE [13]. The suppressed signs of EAE were associated with reduced Th1 and Th17 responses to MOG35–55 in the draining lymph nodes. T cells isolated from the gut-associated lymph nodes showed a selectively suppressed IL-17 production. Interestingly, the effect of antibiotics was not observed in mice whose invariant NKT (iNKT) cells are genetically depleted, indicating the role for iNKT cells in this antibiotic-mediated suppression of EAE. In 2011, Lee et al. [14] reported that although germ-free mice are very resistant against induction of EAE, colonizing the mice with segmented filamentous bacteria (SFB), that is essential for induction of Th17 cells in mice [15], would restore the susceptibility to EAE. Berer et al. used T cell receptor (TCR) transgenic mice that spontaneously develop EAE to address the role of gut microbiota in EAE [16]. They showed that the commensal flora greatly affects the development of disease in their spontaneous EAE model created by genetic engineering.

Analysis of Fecal Samples of MS

These results obtained from rodent EAE experiments indicate a role for the indigenous gut microbiota in the pathogenesis of EAE, and raised the possibility that an altered gut microbiota might be an environmental risk factor for MS. However,

analysis of human fecal commensal microbiota has been a challenge till lately, as the large majority of the gut bacteria is anaerobic and has not been isolated in culture. To overcome the problem, we have used a high-throughput culture-independent pyrosequencing method to compare the gut microbiota of MS patients and healthy subjects [17]. Samples were obtained from 20 patients with relapsing-remitting type of MS during remission and from 40 healthy subjects. In addition, we used 158 control samples from 18 healthy subjects who repeatedly provided the fecal samples.

Bacterial 16S ribosomal RNA (rRNA) gene analysis of fecal DNA revealed that species diversity and richness were not altered in MS (Fig. 10.2). This feature is in striking contrast to the gut microbiota of patients with inflammatory bowel disease (IBD), which is characterized by lower species richness compared with healthy controls. However, UniFrac analysis revealed a significant difference in the overall gut microbiota structure between MS and healthy subjects (Fig. 10.3), indicating that the gut microbiota in MS is significantly altered.

More strikingly, we detected a significant difference in the abundance of 21 bacterial species; two increased and 19 decreased in MS. On comparing MS samples to the 158 longitudinal samples from 18 healthy subjects, the differences were found to be reproducibly significant for most of the species. The taxa reduced in MS comprised primarily of clostridial species belonging to *Clostridia* clusters XIVa and IV and *Bacteroidetes*. Among the reduced clostridial strains, the proportions of *Faecalibacterium prausnitzii* and *Eubacterium rectale* were reduced in fecal and mucosa-associated microbiota in patients with IBD and were associated with a higher risk of postoperative recurrence of ileal Crohn's disease [18, 19].

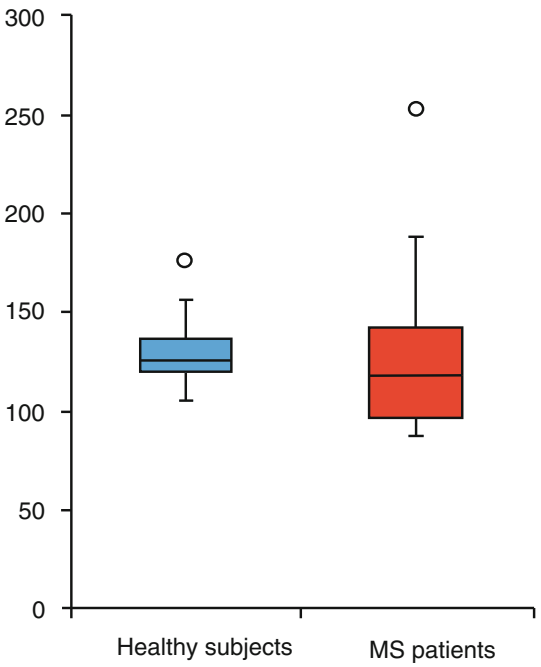

Fig. 10.2 Bacterial species diversity and richness were not altered in MS. Number of operational taxonomic units (OTUs) generated by clustering of 3000 16S reads of gut microbiota samples from 40 healthy control subjects and 20 patients with multiple sclerosis (Revised from Ref. [17])

Clostridial species including *Faecalibacterium prausnitzii* [18] are involved in fermenting digestion of diet fiber, which leads to production of short chain fatty acids (SCFA), including acetate, propionate, and butyrate. Butyrate is known to exert anti-inflammatory functions via inhibition of NF-κB activation and IκB degradation [20]. Interestingly, Atarashi et al. [21] have succeeded in identifying 14 clostridial strains from human feces that are capable of inducing foxp3+ regulatory T cells. Although most of these strains were reduced in IBD samples, they were not phylogenetically close to those that were reduced in MS.

Implications

Results of experimental works as well as epidemiological studies prompted us to evaluate the importance of gut microbiota in MS. As described above, we have found that potentially immunosuppressive clostridial strains are reduced in the gut microbiota from Japanese patients with MS [17]. We could now speculate that the remarkable increase of MS in Japan might result from alterations in the gut microbiota due to the change of lifestyle. If all the bacteria reduced in MS have immune-regulatory potentials like *Faecalibacterium prausnitzii* [18], not only diet therapy but more drastic therapy such as fecal transplantation may deserve consideration for preventive or therapeutic strategies in MS.

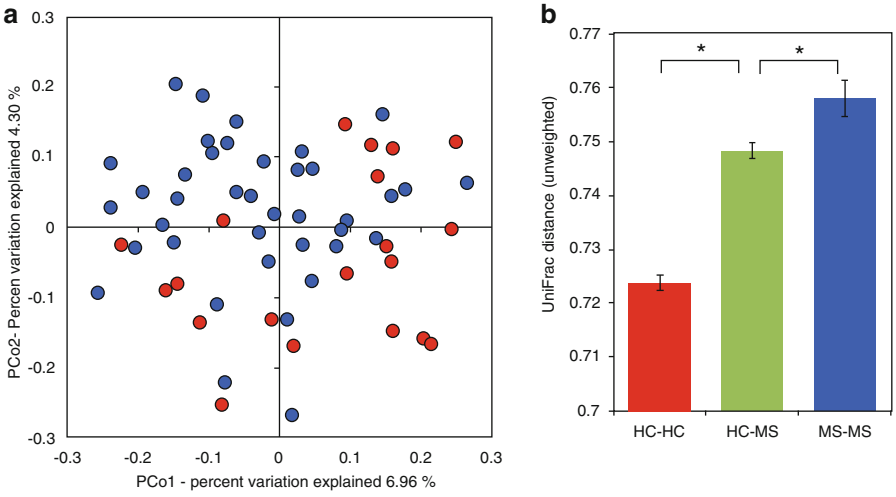

Fig. 10.3 Moderate dysbiosis found in the gut microbiota of MS. Open and closed circles indicate individual subjects from Healthy controls and MS, respectively. (**a**) The two components of the unweighted PCoA plot explained 6.96 and 4.30% of the variance. ANOSIM statistic, $R=0.239$, $P \leq 0.0009$. (**b**) Mean unweighted UniFrac distances for HC-HC, HC-MS, and MS-MS subjects (Revised from Ref. [17])

Gut commensal microbiota are not harmful, but accomplish beneficial functions for promoting and maintaining health. For example, they would serve for hosts through synthesizing vitamins and producing short chain fatty acids (SCFA) with anti-inflammatory activity. Of particular interest, nutritional factors previously reported to show protective effects on MS include vegetable protein, dietary fiber, cereal fiber, vitamin C, thiamin, riboflavin, calcium, and potassium [22]. Dietary fibers are a source of butyrate capable of maintaining intestinal homeostasis. It is also of note that green vegetables contain ligands for aryl hydrocarbon receptor expressed by Th17 cells [23]. Along with rapid progress in basic research, anecdotal or fragmental works, supporting diet therapy of MS, could be now re-evaluated based on more solid scientific background [24, 25]. Ongoing works may lead to development of sophisticated approaches for correcting dysbiosis that may lead to cure of MS in the future.

References

1. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004;4:478–85.
2. Manichanh C, Rigottier-Gois L, Bonnaud E, Pelletier E, Frangeul L, Nalin R, Jrrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut. 2006;55:205–11.
3. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427–34.
4. Peloquin JM, Nguyen DD. The microbiota and inflammatory bowel disease: insights from animal models. Anaerobe. 2013;24:102–6.
5. Chow J, Tang H, Mazmanian SK. Pathobionts of the gastrointestinal microbiota and inflammatory disease. Curr Opin Immunol. 2011;23:473–80.
6. Moreno-Indias I, Cardona F, Tinahones FJ, Queipo-Ortuno MI. Impact of the gut microbiota on the development of obesity and type 2 diabetes mellitus. Front Microbiol. 2014;5:190.
7. Joyce SA, Gahan GM. The gut microbiota and the metabolic health of the host. Curr Opin Gastroenterol. 2014;30:120–7.
8. Yamamura T, Miyake S. Diet, gut flora, and multiple sclerosis: current research and future perspectives. In: Takashi Y, Bruno G, editors. Multiple sclerosis immunology. Heidelberg: Springer; 2013. p. 115–26.
9. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 2002;347:911–20.
10. Houzen H, Niino M, Hirotani M, Fukazawa T, Kikuchi S, Tanaka K, Sasaki H. Increased prevalence, incidence and female predominance in multiple sclerosis in northern Japan. J Neurol Sci. 2012;323:117–22.
11. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: noninfectious factors. Ann Neurol. 2007;61:504–13.
12. Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Muller DN, Hafler DA. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature. 2013;496:518–22.
13. Yokote H, Miyake S, Croxford JL, Oki S, Mizusawa H, Yamamura T. NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. Am J Pathol. 2008;173:1714–23.

14. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2011;108:4615–22.
15. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139:485–98.
16. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, Wekerle H, Krishnamoorthy G. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature. 2011;479:538–41.
17. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim S-W, Morita H, Hattori M, Yamamura T. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to *Clostridia* XIVa and IV clusters. PLoS One. doi:10.1371/journal.pone.0137429.
18. Sokol H, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A. 2008;105:16731–6 (17).
19. Mondot S, Kang S, Furet JP, Aguirre de Carcer D, McSweeney C, Morrison M, et al. Highlighting new phylogenetic specificities of Crohn's disease microbiota. Inflamm Bowel Dis. 2011;17:185–92.
20. Segain J-P, de la Bletiere DR, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottiere HM, Galmiche J-P. Butyrate inhibits inflammatory responses through NFkB inhibition: implications for Crohn's disease. Gut. 2000;47:397–403.
21. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011;331:337–41.
22. Ghadirian P, Jain M, Ducic S, Shatenstein B. Morisset. Nutritional factors in the aetiology of multiple sclerosis: a case-control study in Montreal, Canada. Int J Epidemiol. 1998;27:845–52.
23. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld J-C, Stockinger B. The arylhydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature. 2008;453:106–10.
24. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1:6ra14.
25. David LA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–63.

Chapter 11
Intestinal Parasites and Immunomodulation in Neuroinflammatory Disease

Radu Tanasescu

Abstract Some intestinal parasites are major human pathogens, and deworming is rightly advocated to prevent helminth-induced morbidity. Actual understanding of the immunoregulatory responses induced by helminths, in combination with epidemiological and animal studies, suggests however that intestinal worms may have therapeutic potential in autoimmune diseases such as multiple sclerosis (MS). The epidemiology of MS shows an inverse correlation with helminth infections. Positive effects of helminths in animal models of MS and observational studies in people with MS naturally infected with helminths suggest that those organisms can act as immune regulators and led to clinical trials of helminth therapy. This chapter reviews the animal studies, the rationale for and the safety and efficacy results of clinical trials of helminth therapy in MS. Studies on helminth treatments in MS may provide information that could lead to advances in our understanding of MS pathogenesis.

Keywords Multiple sclerosis • Helminth • Immunoregulation • Hygiene hypothesis • Neuroinflammatory disease

Helminths and Immunoregulation: An Evolutionary Perspective

The ‘hygiene hypothesis’ or ‘microbial deprivation hypothesis’ states that autoimmune and allergic disorders may be an unanticipated consequence of otherwise beneficial advances in sanitation and public health [1–3]. The ‘old friends’ hypothesis is part of the hygiene hypothesis and dwells on the depletion from the urban environment of organisms that accompanied mammalian evolution such as symbiotic

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intestinal microbiota and intestinal worms (helminths) [4, 5]. The 'old friend' organisms stand in contrast to inflammation-promoting new infections acquired by humans during the Neolithic period such as measles, mumps, influenza and smallpox viruses [6]. Helminths had to be tolerated during evolution since their removal by the immune system would translate to unacceptable effects on the host. Therefore, co-evolutionary forces ensured that they came to play essential roles in promoting immunoregulatory pathways involved in tolerance [7]. Autoimmune diseases are more likely in susceptible individuals who have not experienced exposure to 'old friend' organisms such as helminths, as shown by epidemiological surveys which indicate an inverse, dichotomous relationship between helminths and autoimmune conditions [6, 8]. If the absence of helminths is associated with abnormal immunoregulation, the question that arises is whether replacement of helminths will diminish the impact of established autoimmune conditions [6].

Many helminth species mediate protection using similar immune regulatory mechanisms. The mechanisms of protection are not likely to be the same for all autoimmune diseases. The large number of independent regulatory circuits that helminths stimulate may explain why they can influence susceptibility to a number of autoimmune disease states [15, 46]. Common themes include activation of T-regulatory cells (Tregs), induction of regulatory dendritic cells, alteration of macrophage activity and stimulation of regulatory cytokine synthesis (such as IL-10, TGF-β). These mechanisms can function concurrently and independently of each other, and the loss of one regulatory pathway will not necessarily abolish disease protection. Generational exposure to helminths would select in their hosts genetic traits that are suitable for the presence of these organisms. Fumagalli et al. estimated pathogen richness (the number of pathogen species in a specific geographic location) and analysed 91 interleukin (IL) and IL receptor genes (ILR) for 52 human populations distributed worldwide [9]. They showed that helminths have been a major selective force on a subset of these genes, some of them such as IL-1 and ILR-7 being highly relevant to MS [9, 10]. Single nucleotide polymorphisms (SNPs) that display a strong correlation with the diversity of helminth species in different geographic areas map to genes including loci involved in regulatory T-cell function and in macrophage activation, leukocyte integrins and co-inhibitory molecules [11]. Therefore, helminth infection may promote decisive evolutionary effects on the host, especially for genes that control immunoregulation [12]. The absence of this dynamic relationship between helminths and the host recently may have led to an immune incongruity ('biome depletion') [13], and interventions aimed at compensating this mismatch could be a way to prevent or treat autoimmune disease ('biome reconstitution') [13].

Multiple Sclerosis and Natural Infection with Helminths: Epidemiological and Observational Data

The topic of intestinal worms as a therapeutic strategy in multiple sclerosis (MS) has been recently reviewed [8, 12, 14].

MS is a chronic inflammatory disease of the central nervous system (CNS) characterised by faulty immunoregulation. It is considered that MS occurs as a consequence of different gene interactions, abnormal host immune responses and environmental factors. MS incidence has increased in the second half of the twentieth century in developed countries. Fifty years ago, Leibowitz and colleagues were the first to demonstrate that MS was more prevalent in areas of high sanitation [15, 16]. Moreover, current epidemiological evidence shows that MS is spreading into underdeveloped countries where changes in lifestyle occur [17, 18]. *Trichuris trichiura* is a surrogate marker for infection with other macroparasites and low levels of community sanitation, and a prevalence of about 10 % in a given population suggests an exposure to multiple parasite infections [19]. Once a critical threshold in the prevalence of the common human helminth *Trichuris trichiura* is exceeded, the prevalence of MS falls abruptly [19]. The same mutual exclusive tendency of association with MS applies for infections with hookworms (*Necator americanus* and *Ancylostoma duodenale*) [8, 20, 21]. As MS is thought to have a multifactorial pathogenesis, this association may not prove causality, and other factors should be considered (genetics, sunlight exposure, diet) [22–24]. However, this epidemiological evidence may imply either that the absence of intestinal worms increases the risk of MS, that helminths are a marker of a more important aspect directly implicated in the occurrence of MS, or that the intestinal parasites are a protective factor for developing MS [7, 8, 15, 16, 18].

In order for clinical trials of helminth therapy in MS to be designed, conducted and interpreted optimally, it is essential that the interaction between MS and intestinal helminths is understood as well as possible. Gut-based helminths initiate and intervene on the regulation of immune responses at first-contact mucosal and epithelial surfaces. At this first-contact site with the environmental microbiota, the immune system simultaneously discriminates between pathogenic and nonpathogenic microbes and mounts a response protecting the body from the former whilst remaining tolerant of the latter whilst minimising or avoiding damage to the host [6]. Discrimination between microbial pathogens and commensals is facilitated by activation of pattern recognition receptors (PRRs) found on first-contact host cells. Pro-inflammatory molecules are released as a consequence of microbial invasion and cellular stress. Because of the permanent microbial exposure, inflammation is constant in the mucosa; quantitative extraction methods confirm the qualitative histologic impression that the normal gut mucosa is in a state of physiologic inflammation [6]. Immunoregulation, which is influenced by the presence of helminths, would keep this inflammation under control. Therefore, deprivation from intestinal parasites may lead to an excess of pro-inflammatory activity [6].

Important data on helminth-induced immunoregulation in MS are provided by observational studies of MS patients naturally infected with intestinal worms. Those studies suggest that intestinal helminth infection act as an ‘immunological switch’ (turning off MS activity during infection and losing its effects after deworming) [25]. Correale and Farez reported a longitudinal study involving 12 MS patients with mild, asymptomatic intestinal parasite infections, matched with 12 uninfected patients with comparable clinical characteristics [26]. The participants were followed up

for approximately 4.5 years with serial clinical, MRI and immunological assessments. MS patients infected with helminths had a significant reduction in relapses, disability accumulation and new or enlarging T2 lesions or gadolinium-enhancing MRI lesions, downregulation of Smad7 signalling with increased numbers and activity of Treg and a parasite-induced Th2-type cytokine response in comparison with the uninfected control patients [26]. Mechanistic studies demonstrated that the improved control of MS in the infected group was associated with cellular immune responses characterised by increases in IL-10 and TGF-β expression, decreases in IL-12 and expression of IFN-γ and also induction B-regulatory cells [26–28]. Moreover, helminth-related immunomodulation observed in MS patients was mediated by Toll-like receptor (TLR) 2 and retinoic acid-dependent pathways via induction of IL-10 and Treg and suppression of pro-inflammatory cytokine production mediated by suppressor of cytokine signalling 3 (SOCS3) [29].

More data were reported from the 7.5-year follow-up of the demographically matched groups of infected and uninfected MS patients and healthy controls (HC) (12 in each group) [30]. During the first 5 years of follow-up, the infected group had a significantly lower disease activity than the uninfected group. After 5.25 years, four MS patients infected with helminths were dewormed. After helminth eradication, MS clinical and radiological activities increased to the level seen in the uninfected group. Mechanistic immunological studies of peripheral blood mononuclear cell responses after stimulation with myelin basic protein or phytohaemagglutinin showed that the MS patients had increased IL-12- and IFN-γ-secreting cells and lower numbers of IL-10- and TGF-β-secreting cells in comparison with HC. This pattern, as well as Treg numbers, reversed during helminth infection but returned to a pro-inflammatory state after deworming. The limitations of the study were the lack of blinding, the small size and the observational design [25].

Helminths Treatment in the Animal Model of MS

The most commonly studied animal model of MS is experimental autoimmune encephalitis (EAE). EAE mimics several of the key clinical and pathological features of MS and is a proven tool of testing therapies effective in human studies [31–35]. Nevertheless, EAE models the effects of an intervention for an individual inflammatory attack and provides less information on the long-term impact that an intervention could have on the course of a recurring conditions such as MS [8]. Since many models of EAE are monophasic in contrast to MS, helminth treatment in people with MS is likely to require continuous treatment [12].

Parasite effects on EAE have been recently reviewed and compared to EAE studies involving protozoan organisms [12, 36]. Data generated from these EAE studies vary broadly depending on study designs, schedules of administration, types and doses of specific live helminths or helminth products, sites of action and type of pathological changes of host tissue and alterations in biomarkers [12]. However, the overwhelming majority of EAE studies showed that treatment with live helminths or helminth products has beneficial effects in EAE [37–50] (Table 11.1), whilst one study did not show any effect [49].

Table 11.1 Experimental studies of helminth therapy in EAE

Animal model[a]	Species, strain[b]	Helminth treatment[c]	Effect in which helminth was administered depending on[d] phase EAE severity			Mechanism of effect, comments[e]	Reference
			Pre-immunisation	Induction	Effector		
Live helminth administration							
EAE $MOG_{35–55}$	Mouse C57BL/6J	*S. mansoni* (trematode systemic/hepatic) 70 cercariae cutaneous Gastrointestinal colonisation	++			Reduction of Th1 pro-inflammatory cytokines	La Flamme et al. [39]
EAE $MOG_{35–55}$	Mouse C57Bl/6	*Fasciola hepatica* (trematode, hepatic) ten metacercariae p.o. Liver colonisation	++			Bystander attenuation of Th17 and Th1 responses by means of TGF-β	Walsh et al. [45]
EAE spinal cord homogenate	Rat DA female	*Trichinella spiralis* (nematode, systemic) larvae, gastric Muscle colonisation	+	++		Th2 cytokine bias; also, anti-inflammatory responses likely due to CD4+CD25+Foxp3+ regulatory cells	Gruden-Movsesijan et al. [48]
EAE $MOG_{35–55}$	Mouse C57BL/6 female	*Trichinella pseudospiralis* (nematode, systemic) 200 larvae p.o. Muscle colonisation	++			Strong Th2 responses; suppressed Th1 and Th17 responses	Wu et al. [44]
EAE $MOG_{35–55}$	Mouse C57BL/6	*Heligmosomoides polygyrus* (nematode, enteric) 200 larvae gavage Gut mucosal and submucosa	+++	++		In adoptive transfers, both CD4+ (T regulatory) and CD4 (B regulatory?) cells ameliorate disease	Wilson et al. [46]

(continued)

Table 11.1 (continued)

Animal model[a]	Species, strain[b]	Helminth treatment[c]	Effect in which helminth was administered depending on[d] phase EAE severity			Mechanism of effect, comments[e]	Reference
			Pre-immunisation	Induction	Effector		
EAE $MOG_{35–55}$	Mouse C57Bl/6; female	*Taenia crassiceps* (cestode, gastrointestinal and systemic) 40 metacestodes, i.p. Peritoneal cavity colonisation				Anti-inflammatory cytokine environment leads to reduced T-cell activation, proliferation and migration into the CNS	Reyes et al. [47]
EAE MBP	Rat Lewis female	*Strongyloides venezuelensis* (nematode, gastrointestinal) 4,000 larvae s.c. Gastrointestinal colonisation	–			No effect on disease course; possibly this host is non-permissive for sustained *S. venezuelensis* infection	Chiuso-Minicucci et al. [49]
Helminth product administration							
$^{EAE\ PLP}135–151$	Mouse C57BL/6, SJL female	*Schistosoma mansoni* (trematode systemic/hepatic) 5,000–20,000 eggs i.p. Peritoneal granulomas	++	+	–	Polarisation of immune responses, creating a Th2 environment	Sewell et al. [37]
EAE $MOG_{35–55}$	Mouse C57Bl/6 female	Soluble egg antigen (SEA) from *Schistosoma japonicum* (trematode, systemic/hepatic) Multiple doses of 100 l g SEA i.p.	+	++		Th2 environment established	Zheng et al. [38]

EAE $MOG_{35–55}$	Mouse C57Bl/6 female and human dendritic cells	Soluble products (SP) from three different helminths, multiple doses of 100 l g SP i.p. *Trichuris suis* (nematode, gastrointestinal), or *Trichuris spiralis* (nematode, systemic), or *S. mansoni* (trematode systemic/ hepatic)	++			Helminth SP suppress pro-inflammatory cytokines In vitro, incubation of human dendritic cells with helminth SP leads to phenotype which suppresses memory T-cell IL-17 production and promotes Th2 polarisation Effects and mechanisms varied somewhat with different helminth SP (see publication for details)	Kuijk et al. [40]
EAE $MOG_{35–55}$	Mouse C57Bl/6 female	LNFPIII synthetic glycan from *S. mansoni*[f] (trematode, systemic/hepatic) Multiple doses of 50 l g i.p		++		LNFPIII molecule modulates Th1/ Th2 balance, T-cell regulation by inflammatory monocytes and dendritic cell migration	Zhu et al. [43]
EAE rat spinal cord tissue homogenate	DA rats, male	In vivo effect of ES L1 *Trichinella spiralis* antigen-educated DCs administrated prior to induction on the development of EAE	+	+	+	ES antigen-stimulated DCs are able to initiate and sustain anti-inflammatory and regulatory responses regardless of EAE induction, with subsequent amelioration of EAE. Increased production of IL-4, IL-10 and TGF-β, decreased production of IFN-γ and IL-17, increase in Treg both at the systemic level and in target organs	Sofronic-Milosavljevic et al. [42]

(continued)

Table 11.1 (continued)

EAE $MOG_{35–55}$	Mouse C57Bl/6 female	*Heligmosomoides polygyrus* 200 larvae administered after 21 days			+	Post-established infection of *H. polygyrus* in L4 stage can inhibit ongoing EAE: reduction in EAE symptoms observed from 2 days postinfection; symptoms almost completely inhibited at 6 days post infection	Donskow-Łysoniewska et al. [41]
EAE rat spinal cord tissue homogenate	DA rats	*Trichinella spiralis* ES L1 before EAE induction	+	–	–	ES L1 products ameliorates EAE. increased proportion of unconventional CD4+CD25-Foxp3+ regulatory cells both in the periphery and in the CNS	Radovic et al [50]

Modified with permission from [8]

PLP proteolipid protein, *MOG* myelin oligodendrocyte glycoprotein, *p.o.* oral administration

[a]The animal model of MS employed in these studies was experimental autoimmune encephalomyelitis (EAE). The specific neuroantigen with which animals were immunised is indicated for each study

[b]The strain of each animal in which EAE was induced is shown, together with the animals' sex, if indicated in the original publication

[c]For each study of helminth therapy in EAE, the following are summarised in order: helminth species, informal medical classification by phylum or class and usual tropism of this type of worm for either specific organs or generalised systemic distribution, dose and route of helminth administration and the site/type of helminth colonisation or infection in the specific experiment. Studies are divided into those in which either live parasites or purified helminth products were used for treatment. In some studies complex- or multiple-dosing schedules were applied; see original publications for details

[d]The effect of helminth treatment in EAE is shown by the phase of the model disease, depending on the timing of the helminth administration or action, that is, helminth given before neuroantigen immunisation (pre-immunisation phase of EAE), shortly after neuroantigen immunisation (0–10 days, induction phase of EAE) or later after neuroantigen immunisation (10–30 days, effector phase of EAE). The approximate benefit of treatment on EAE severity, depending on the time at which helminth was administered is indicated by the following symbols: no effect; +, moderate; ++, strong; and +++, dramatic. If no symbol is shown, the investigation did not address the effects of helminth treatment at the indicated phase

[e]The general or predominant mechanism of helminth therapeutic effects in the EAE model is briefly summarised for each study. Because the experimental design, assays, results and mechanisms of action are complex, the original reports should be consulted for details and clarifications

[f]The derivation and terminology for helminth-expressed glycans are complex; subsequent studies refine the characterisation of the molecule in this study to the Lewis X glycan [51]

Administration of helminths has different effects depending on the stage of EAE. In all but two of the EAE studies in which the treatment induced changes [41, 42], the more localised helminthic activity was mostly effective in the pre-immunisation and induction phases of EAE. Treatment with soluble egg antigen from *Schistosoma* spp. only had an effect when administered before induction of EAE [38]. This suggests that in order to be effective, helminth treatments should be administered early, before irreversible damage of the CNS occurs.

Helminth treatment in EAE induces a general anti-inflammatory milieu through multiple pathways that finally regulate the activity of autoreactive T cells and effector cells [8]. Firstly, helminths generate a modified Th2 response with high amounts of IL-4, IL-5, IL-10 and distinct IgG subclasses as well as an increase in Tregs. The majority of studies of helminth treatment in EAE show the induction of a Th2 profile with an increase of IL-4 and IL-5 and a decrease of IFN-γ, IL-12 and IL-17 [8, 41]. The positive effects on EAE outcome are unlikely to be mediated only by a Th1-Th2 shift. Sewell et al. showed no effect of helminth treatment on EAE severity in STAT6 deficient mice, a key regulator of Th2 differentiation [37]. Moreover, a Th2 shift was induced also in the only published EAE study that failed to show a positive effect of helminth treatment following infection [49]. Importantly, the percentage and absolute number of Treg cells (CD4 + CD25 + Foxp3+ T cells) were not changed, suggesting that a Th2-polarised response without concomitant expansion of Treg was not enough to modify EAE outcome [49].

Secondly, induction of Treg is typically seen in conjunction with increased IL-10 and TGF-β [52, 53]. Although IL-10 primarily suppresses local helminth-specific T-helper cell responses such as production of IL-4, IFN-γ and IL-17 [45], it is not a primary modulator of the autoimmune response. IL-10 knockout mice with EAE and infected with helminths exhibit similar reductions in clinical severity as wild-type mice [45]. Adoptive transfer of mesenteric lymph node cells from helminth-infected mice in EAE and allergic disease influences disease outcomes equally in animals that received cells from IL-10-negative or wild-type mice [46, 52]. Whilst IL-10 plays a central role in protozoan-mediated immune suppression [54, 55], other mediators such as TGF-β seem to be crucial for helminth-induced suppression. In helminth-infected EAE animals, the disease is restored by neutralisation of TGF-β [45]. TGF-β reduces production of pro-inflammatory cytokines and controls differentiation of alternatively activated macrophages [56, 57]. In animals with EAE and helminth infection, this macrophage phenotype is associated with decreased disease activity [47]. In parasite-infected animals, there is an increased production of TGF-β by isolated splenocytes, concomitant with Treg induction [36]. Moreover, TGF-β can be produced by Treg cells, and it further influences their differentiation [57, 58].

Under the influence of helminth excretory-secretory (ES) products, dendritic cells (DCs) may acquire a semi-mature phenotype and are able to polarise naive T cells in vitro and in vivo [42]. When ES-pre-stimulated DCs were used to pretreat animals undergoing EAE induction, they were able to reduce the clinical signs and the duration of the disease. Treated animals had decreased production of IFN-γ and IL-17 and increased production of IL-4, IL-10 and TGF-β, as well as an expansion of Treg in the spinal cord and spleen [42]. In a recent study, prophylactic application

of *Trichinella spiralis* excretory-secretory muscle larvae products ameliorated EAE with the same success as infection did [50]. However, additional to the shift to the Th2-type response, the authors noted an increased proportion of unconventional CD4+CD25-Foxp3+ Tregs both in the periphery and in the CNS of animals treated with ES L1 before the induction of EAE [50].

To conclude, treatment of EAE with helminths generates an immunoregulatory response which involves tolerising stimulation of B cells and DC by helminth-derived molecules, induction of Tregs and production of TGF-β and IL-10. Those effects are beyond the classical Th2 response and could explain why helminth treatment modulates both Th1- and Th2-driven conditions [8].

Helminths in Clinical Trials in MS

Principles of and recommendation for trials of helminth therapy in autoimmune disease have been recently published [6]. The two helminth species used in clinical trials of helminth therapy in MS were *Trichuris suis* and *Necator americanus*; both are chosen due to their favourable safety profile in a setting of controlled infection.

Most of the therapeutic helminth trials in MS have used *Trichuris suis*, the porcine whipworm. *Trichuris suis* is closely related to *Trichuris trichiura* (human whipworm) and can briefly colonise people [59]. Microscopic parasite eggs are ingested, and each egg releases one larva that matures into an adult worm. The features that make *Trichuris* species good candidates for clinical use are the following [8]: (1) larvae and adults do not migrate beyond the gut and do not multiply within the host; (2) *Trichuris suis* has not been documented to cause human disease; (3) normal hygienic practices impede transmission from host to host, since ova require incubation in moist soil for 1–2 months to mature and become infective; and (4) *Trichuris suis* obtained from pigs is cultured in a specific pathogen-free environment; thus, any risk from using this helminth is likely to be small [8]. The downsides regarding the use of *Trichuris suis* are the following: (1) the parasite is zoonotic; therefore errant migration to various organs in the human host cannot be excluded; (2) infection is short-lived (2 weeks) requiring frequent dosing; and (3) financial cost of frequent dosing is high [60].

The hookworm *Necator americanus* is a gastrointestinal pathogen that infects over 500 million people. The parasite is encountered only in humans, which makes it a 'family heritage' and an evolutionary 'old friend' that has accompanied humans during historical migration [8]. Infection with *Necator americanus* is generally benign once adult worms are established in the gut; however it can produce anaemia if infection intensity is heavy or if iron status is compromised [61–63]. In hookworm-endemic populations, the hookworm induces a mixed peripheral T-helper cell response with Th2, IL-10 and TGF-β dominance [64, 65]. For clinical application, people are colonised by applying infective larvae to the skin. This method mimics natural infection, which occurs after the subject walks barefoot on the larvae that have hatched after incubation on the soil [8]. After penetrating the intact skin of the human, the larvae

migrate to the lungs, enter the bronchi and migrate up the trachea to the throat where they are swallowed residing in the small gut and maturing. To obtain larvae for therapeutic use, they are cultured from the stool of human volunteer donors that are actively colonised with *Necator americanus* and screened to reduce the risk of transmitting other infections [7]. After the larvae are washed to eliminate any bacterial co-infection, they are applied to the skin [8]. The advantages of using this helminth are that the hookworm establishes a chronic but localised infection that can last more than 5 years [66] and the systemic exposure created by larval migration may be more effective at activating a range of different immune compartments [8].

The theoretical drawbacks of *Necator americanus* treatment are pulmonary damage during larval transit, anaemia due to gastrointestinal blood loss and altered airway responsiveness. However, studies at the University of Nottingham have shown that controlled infection with a small number of larvae is very safe and does not have any pulmonary or haematological side effects [67]. Acute infection can cause gastrointestinal symptoms, but dose-ranging studies showed that light infection (e.g. ten larvae) is asymptomatic [68–70]. A successful parasite-host relationship is one that edges on commensalism, where the parasite causes little-to-no overt damage to its host, and ideally approaches mutualism, where the host actually derives some benefit from the parasite [60, 63, 71]. *Necator americanus* would fit this profile for its potential benefits in treating MS or other chronic diseases of inflammation [71]. The safety and therapeutic effect of low dose of *Necator americanus* infection has been evaluated for a number of inflammatory diseases, proving to be safe and tolerable [60, 62, 65, 67, 72–74].

Clinical trials of helminth therapy in MS have been recently reviewed [8]. The first phase I clinical trial of helminth therapy in MS (the HINT study: Helminth-induced immunomodulation therapy) was conducted by Fleming et al. at the University of Wisconsin, USA [75]. It followed preclinical studies conducted between 2005 and 2007 [12]. In the first part of the trial (HINT 1), five relapsing-remitting MS subjects were treated with 2,500 live *Trichuris suis* ova (TSO) orally every 2 weeks for 3 months [75]. TSO were microbiologically checked by the producer and at the University of Wisconsin for all porcine adventitious agents and other microbiological contaminants [12, 75]. Brain MRI was performed at baseline, monthly for 3 months and at 2 months after the end of TSO treatment. The mean number of new active brain lesions was 6.6 at baseline, 2.0 after 3 months of treatment, and 5.8 at 2 months post-treatment. The authors noted that the promising MRI results should be interpreted with caution, given the small number of subjects and the short period of observation [75]. No major adverse clinical effects were reported in the HINT 1 subjects. In three of the five subjects, transient mild gastrointestinal symptoms that did not interfere with daily living activities were reported at approximately 30 days after TSO initiation. This ‘first-dose’ phenomenon was similar to that reported in a study of TSO for allergic rhinitis [76]. Biologically, TSO treatment resulted in eosinophilia, an elevation of serum C-reactive protein and antibody to *Trichuris suis* ES products (IgG1, IgA, but not IgE) and an increase in serum IL-4 and IL-10. TSO therapy produced changes suggestive of modulation of TLR regulatory pathways, but didn’t have an effect on the percentage of circulating monocytes

expressing typical surface markers of alternatively activated macrophages from the PBMCs of treated patients when compared to healthy controls [12]. This lack of change suggested that any alternatively activated macrophage-inducing soluble factors at the site of helminthic infection, if present, had no effect on the phenotype of circulating monocytes [25].

HINT 2 was a follow-up exploratory clinical trial with baseline versus treatment design involved 15 treatment-naive relapsing-remitting MS [12, 77]. The patients underwent 5 months of pretreatment observation and 10 months of treatment with *Trichuris suis* ova (2,500 live ova orally every 2 weeks). The primary outcome measures were the safety and tolerability of *Trichuris suis* ova and the change in the number of gadolinium-enhancing lesions (Gd+) during monthly brain MRI scans with double-dose gadolinium contrast [12]. No significant safety or tolerability issues were observed. The mean number of Gd+lesions per month was 3.2 during 5 months of observation and 2.1 during the last 5 months of treatment, i.e. a 34% relative reduction. Immunological assessments indicated that TSO was associated with increases in Treg cells and a modified Th2 immune response. Transcriptional analyses of peripheral blood mononuclear cells suggested that treatment led to diminished expression of the pellino E3 ubiquitin protein ligase 1 (pelli 1) gene, recently demonstrated to be a central activator of microglia in experimental autoimmune encephalomyelitis and possibly in MS itself [78]. The investigators concluded that TSO appears safe and well tolerated in RRMS subjects and that the modest decrease observed in numbers of Gd+lesions during treatment indicates that further studies of TSO will be required to assess its effectiveness in RRMS [79].

A pilot, exploratory study of helminth therapy in secondary progressive MS (SPMS) was conducted by Benzel et al. at the Charite University, Berlin, Germany [80]. Four SPMS subjects were treated for 6 months with 2,500 TSO administered orally every 2 weeks. The patients were clinically stable during the study, and treatment was well tolerated [80]. To determine whether TSO limits the CD4+Th1 response or instead increases the general Th2 response, they stimulated whole blood cells with different superantigens (staphylococcus enterotoxins A and B and toxic shock syndrome toxin) before, during and after therapy with TSO in vitro and subsequently stained them for CD154, CD4, IFN-g, IL-2, IL-4 and IL-10. Immunological monitoring showed a slight downregulation of the Th1-associated cytokine pattern, especially IL-2, with a temporary increase of Th2-associated cytokines such as IL-4 [80]. Mild eosinophilia and changes in CD4+ and CD8+ T cells and natural killer (NK) CD56 bright cell numbers were observed. Stimulated PBMC showed a trend towards an initial increase of IL-2 and IFN-γ after 1 month, followed by a reduction in these cytokines after 2 months [80]. This suggests an early pro-inflammatory response to the helminth infection followed by an anti-inflammatory Th2 response, as previously described [81, 82]. A significant decrease of serum brain-derived neurotrophic factor (BDNF) levels during TSO therapy was reported [83]. This was different from reports from naturally infected patients, in which an increased production of BDNF and nerve growth factor in stimulated B cells from MS patients with a

helminth infection compared to uninfected patients and controls was reported [28]. Several differences in study design were suggested as explanations for the opposite trends in BDNF levels (RRMS vs. SPMS, stimulated B cells vs. serum levels, natural infections vs. experimental TSO treatment, clinical observational vs. prospective clinical trial study design) [83].

Rosche et al. have initiated a phase II study aiming to enrol 50 RRMS subjects who will be treated with either TSO or placebo for 12 months (*Trichuris suis* ova in relapsing-remitting multiple sclerosis (TRIOMS) and clinically isolated syndrome) [84]. In comparison to HINT2, TRIOMS includes a placebo-controlled arm, and it aims to include more patients. The study is currently ongoing. As in HINT 2, the safety, tolerability and effect on disease activity and in vivo mechanisms of action of TSO in MS will be assessed by neurological, laboratory and immunological exams and MRI throughout the 12-month treatment period and over a follow-up period of 6 months [84]. PBMCs from the peripheral blood will be sampled prior to and during the intervention to assess the effect of TSO treatment on cellular and soluble components of the immune system (Table 11.2).

Voldsgaard et al. conducted an open-label, MRI assessor-blinded safety study of ten RRMS patients treated with 2,500 TSO orally for 3 months (TRIMS A) [85]. Six from ten patients were concomitantly treated with β-interferon. MRI was performed every 3 weeks. The investigators concluded that TSO was safe and well tolerated but that no clinical, MRI or immunological signals suggestive of a benefit were observed [85]. The trial design was adapted to test safety and not drug effectiveness. The concomitancy of disease-modifying therapies in more than half of the patients, the small patient sample and the short follow-up do not allow any conclusions in terms of effectiveness of helminth therapy in this study [8].

The first phase II double-blinded placebo-controlled clinical trial of hookworm treatment in relapsing MS is currently ongoing at the University of Nottingham (Worms for Immune Regulation of MS (WIRMS)) [8, 86]. Seventy-two RRMS patients will be treated either with 25 dermally administered hookworm (*Necator americanus*) larvae or with placebo. In order to be included, patients should be between 18 and 65 years old, should have at least one relapse in the last 12 months or two in the last 24 months and an expanded disability status scale (EDSS) score in the range of 0–5.5 at baseline [8]. The primary endpoint consists in the cumulative number of new or enlarging Gd+lesions at 9 months, whilst several immunological parameters reflecting Treg expression and activity and Th2 shift are secondary and exploratory outcome measures. MRI scans are performed monthly from 3 months to 9 months and 3 months after deworming [8]. Interim safety analysis as per January 2015 suggests good tolerability and safety profile of treatment in this trial.

In short, pilot MS studies with helminths have shown a very good safety profile and some encouraging effects on clinical, radiological and immunological outcomes. Results from phase II studies are needed in order to confirm the promising hints suggested by preclinical, epidemiological and observational and pilot therapeutic studies regarding effectiveness of helminth therapies in MS [8].

Table 11.2 Clinical studies of helminth therapy in multiple sclerosis

Type of investigation[a]	Study[b]	Status[c]	Subjects[d]	Helminth[e]	ClinicalTrials.gov[f]	Comments	Publications[g]
Observational							
	Correale and Farez	C	12 RRMS	Natural gastrointestinal infections with human helminths		Dramatic reduction in clinical, MRI and immunological measures of MS activity found	Correale and Farez [26, 30]
Exploratory							
	HINT 1	C	5 RRMS	*Trichuris suis* ova 2,500 q 2 weeks × 12 weeks orally	NCT00645749	Treatment was safe; MRI and immunological outcomes favourable	Fleming et al. [75]
	Charite	C	4 SPMS	*Trichuris suis* ova 2,500 q 2 weeks × 24 weeks orally		Treatment was safe; moderate positive immunomodulatory impact	Benzel et al. [80]
	TRIMS A	C	10 RRMS	*Trichuris suis* ova 2,500 q 2 weeks × 12 weeks orally	NCT01006941	Treatment was safe; no clinical, MRI and immunological benefit	AAN (2012)
Phase 1/2							
	HINT 2	P	15 RRMS	*Trichuris suis* ova 2,500 q 2 weeks × 10 months orally	NCT00645749	Safety confirmed; interim MRI and immunological measures positive; final results expected 2014	AAN (2012), ACTRIMS (2012)

	TRIOMS	P	50 RRMS	*Trichuris suis* ova 2,500 q vs placebo 2 weeks × 12 months orally	NCT01413243	Trial recruiting	
	WIRMS	P	72 RRMS	25 live *Necator americanus* dermally (single administration) vs placebo; 9 month follow-up of infection effects	NCT01470521	Trial recruiting	Edwards and Constantinescu (2009)

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MRI magnetic resonance imaging
[a]Studies are classified as observational (field study, naturally acquired infections), exploratory (preliminary pilot first-use safety clinical trials) or early phases I–II (follow-up clinical trials)
[b]Each study is designated by location, investigators or acronym
[c]Study status is indicated by C (completed), P (in progress or enrolling) or A (initiation anticipated in the near future)
[d]The number and type of subjects are noted (*RRMS* relapsing-remitting MS, *SPMS* secondary progressive MS); only the number of subjects with MS are shown, exclusive of subjects in placebo or observational arms
[e]The helminth infection or treatment indicated by agent, dose, duration and route
[f]Clinical trial listings are provided by study number on the clinicaltrials.gov website; these listings provide details of study design and periodic updates on study progress
[g]Publications or meeting presentations (*AAN* American Academy of Neurology, 2012, *ACTRIMS* Americas Committee for Treatment and Research in Multiple Sclerosis, 2012) are indicated

Difficulties of Therapeutic Studies with Helminth and Future Directions

Fleming and Weinstock have recently highlighted some of the issues raised by the current clinical studies of helminth treatments [6]. The early studies included small numbers of subjects and limited resources. However, the purposes of those exploratory trials were to evaluate initial safety and to determine whether sufficient potential exists to warrant larger follow-up studies [6]. Patient selection (too mild or too severe disease), the dose and schedule of helminth treatment and statistical power issues are to be considered when designing a clinical trial with intestinal parasites in autoimmune disease, including MS [6]. An important point remains the choice and manufacturing of the helminth agent, which may alter the longevity and vitality of the parasite [6]. It may be possible that only a human pathogen may be therapeutic in human autoimmune disease or that the control of chronic condition requires purified helminth-derived immune regulatory molecules administered at different time points [6]. An important point regards the possibility that patients with MS raised in a hygienic or Western environment may not have significant childhood exposures to helminths [87]. Thus, they may not have 'the type of childhood epigenetic imprinting which in adulthood will be adequate to support a strong immune regulatory response upon therapeutic application of helminths' [6]. This would influence their immunological response during a trial using helminths which would be different from the response seen in people raised in areas where helminths are endemic, as was the case in naturally infected MS patients in the observational studies [26]. This latter group may generate a robust immunoregulatory response to helminth treatment [6]. Moreover, it is possible that once full-blown MS and associated secondary effects such as epitope spreading occur [88], an absolute control may not be achievable. Therefore, research on parasite influence on MS should ideally tackle the preclinical stages of MS. Finally, the hygiene hypothesis may be incorrect, and the observed inverse relationship of MS and helminths may prove to be just a marker for a more fundamental process in the pathogenesis of MS involving many factors (e.g. UV light exposure, vitamin D, inciting infection, environmental toxins [6]).

Deworming is rightly advocated to prevent helminth-induced morbidity; however it may lead to the emergence of metabolic and inflammatory conditions including MS, in countries that are not prepared for these new epidemics [89]. Further studies are needed to assess this risk and to enhance understanding of how helminths modulate inflammatory and metabolic pathways [89].

The relationship between MS, and parasites and infections in general is complex and unclear. The types and timing of particular microbial exposures, the character of innate immune responses they induce and the downstream effects of continued microbial pressure from the innate immune system on the adaptive immune system may all play a potential role in MS immune dysregulation [6]. Data from clinical trials and animal and basic science studies which address the mechanism of helminth exposure in MS are important not only for a better management of MS but also by unravelling the relationship between intestinal parasites and autoimmunity in general.

References

1. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299(6710):1259–60. Pubmed Central PMCID: 1838109.
2. Bjorksten B. The hygiene hypothesis: do we still believe in it? Nestle Nutr Workshop Ser Paediatr Program. 2009;64:11–8; discussion 8–22, 251–7.
3. Kemp A, Bjorksten B. Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol. 2003;14(2):74–80.
4. Rook GA. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42(1):5–15.
5. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol. 2010;160(1):1–9. Pubmed Central PMCID: 2841828.
6. Fleming JO, Weinstock JV. Clinical trials of helminth therapy in autoimmune diseases: rationale and findings. Parasite Immunol. 2015;37(6):277–92.
7. Elliott DE, Weinstock JV. Helminthic therapy: using worms to treat immune-mediated disease. Adv Exp Med Biol. 2009;666:157–66. PubMed PMID: WOS: 000271245900012. English.
8. Tanasescu R, Constantinescu CS. Helminth therapy for MS. Curr Top Behav Neurosci. 2015;26:195–220. doi: 10.1007/7854_2014_361.
9. Fumagalli M, Pozzoli U, Cagliani R, Comi GP, Riva S, Clerici M, et al. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J Exp Med. 2009;206(6):1395–408. PubMed PMID: WOS: 000267133700017. English.
10. Lundmark F, Duvefelt K, Iacobaeus E, Kockum I, Wallstrom E, Khademi M, et al. Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat Genet. 2007;39(9):1108–13.
11. Fumagalli M, Pozzoli U, Cagliani R, Comi GP, Bresolin N, Clerici M, et al. The landscape of human genes involved in the immune response to parasitic worms. BMC Evol Biol. 2010;10:264. PubMed PMID: WOS: 000282768700002. English.
12. Fleming JO. Helminth therapy and multiple sclerosis. Int J Parasitol. 2013;43(3–4):259–74.
13. Parker W, Ollerton J. Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. Evol Med Public Health. 2013;2013(1):89–103. Pubmed Central PMCID: 3868394.
14. Correale J. Helminth/Parasite treatment of multiple sclerosis. Curr Treat Options Neurol. 2014;16(6):296.
15. Alter M, Leibowitz U, Halpern L. Multiple sclerosis in European & Afro-Asian populations of Israel. A clinical appraisal. Acta Neurol Scand. 1966;42 Suppl 19:47–54.
16. Leibowitz U, Antonovsky A, Medalie JM, Smith HA, Halpern L, Alter M. Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and level of sanitation. J Neurol Neurosurg Psychiatry. 1966;29(1):60–8. Pubmed Central PMCID: 495985.
17. Koch-Henriksen N, Sorensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 2010;9(5):520–32.
18. Koch-Henriksen N, Sorensen PS. Why does the north–south gradient of incidence of multiple sclerosis seem to have disappeared on the Northern hemisphere? J Neurol Sci. 2011;311(1–2):58–63. PubMed PMID: WOS: 000297893400010. English.
19. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis. Neurology. 2006;67(11):2085–6. PubMed PMID: WOS: 000242750900042. English.
20. Hotez PJ, Bethony J, Bottazzi ME, Brooker S, Buss P. Hookworm: "the great infection of mankind". Plos Med. 2005;2(3):187–91. PubMed PMID: WOS: 000228382500005. English.
21. http://www.msif.org/includes/documents/cm_docs/2013/m/msif-atlas-of-ms-2013-report.pdf. 2013.
22. Sawcer S. The major cause of multiple sclerosis is environmental: genetics has a minor role – no. Mult Scler J. 2011;17(10):1174–5. PubMed PMID: WOS: 000295695100006. English.

23. Hutchinson M. The major cause of multiple sclerosis is environmental: genetics has a minor role – commentary. Mult Scler J. 2011;17(10):1176. PubMed PMID: WOS: 000295695100007. English.
24. Taylor BV. The major cause of multiple sclerosis is environmental: genetics has a minor role – yes. Mult Scler J. 2011;17(10):1171–3. PubMed PMID: WOS: 000295695100005. English.
25. Fleming JO. Helminths and multiple sclerosis: will old friends give us new treatments for MS? J Neuroimmunol. 2011;233(1–2):3–5.
26. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol. 2007;61(2):97–108.
27. Correale J, Farez M. Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. J Immunol. 2009;183(9):5999–6012.
28. Correale J, Farez M, Razzitte G. Helminth infections associated with multiple sclerosis induce regulatory B cells. Ann Neurol. 2008;64(2):187–99.
29. Correale J, Farez MF. Parasite infections in multiple sclerosis modulate immune responses through a retinoic acid-dependent pathway. J Immunol. 2013;191(7):3827–37.
30. Correale J, Farez MF. The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol. 2011;233(1–2):6–11.
31. Kuerten S, Angelov DN. Comparing the CNS morphology and immunobiology of different EAE models in C57BL/6 mice – a step towards understanding the complexity of multiple sclerosis. Ann Anat = Anatomischer Anzeiger: Off Organ Anatomische Gesellschaft. 2008; 190(1):1–15.
32. Mix E, Meyer-Rienecker H, Hartung HP, Zettl UK. Animal models of multiple sclerosis–potentials and limitations. Prog Neurobiol. 2010;92(3):386–404.
33. t Hart BA BA, Gran B, Weissert R. EAE: imperfect but useful models of multiple sclerosis. Trends Mol Med. 2011;17(3):119–25.
34. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011;164(4):1079–106. Pubmed Central PMCID: 3229753.
35. Farooqi N, Gran B, Constantinescu CS. Are current disease-modifying therapeutics in multiple sclerosis justified on the basis of studies in experimental autoimmune encephalomyelitis? J Neurochem. 2010;115(4):829–44.
36. Hasseldam H, Hansen CS, Johansen FF. Immunomodulatory effects of helminths and protozoa in multiple sclerosis and experimental autoimmune encephalomyelitis. Parasite Immunol. 2013;35(3–4):103–8.
37. Sewell D, Qing Z, Reinke E, Elliot D, Weinstock J, Sandor M, et al. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. Int Immunol. 2003;15(1):59–69.
38. Zheng X, Hu X, Zhou G, Lu Z, Qiu W, Bao J, et al. Soluble egg antigen from Schistosoma japonicum modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response. J Neuroimmunol. 2008;194(1–2):107–14.
39. La Flamme AC, Ruddenklau K, Backstrom BT. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect Immun. 2003;71(9):4996–5004. Pubmed Central PMCID: 187318.
40. Kuijk LM, Klaver EJ, Kooij G, van der Pol SM, Heijnen P, Bruijns SC, et al. Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis and differentially modulate human dendritic cell activation. Mol Immunol. 2012;51(2):210–8.
41. Donskow-Lysoniewska K, Krawczak K, Doligalska M. Heligmosomoides polygyrus: EAE remission is correlated with different systemic cytokine profiles provoked by L4 and adult nematodes. Exp Parasitol. 2012;132(2):243–8. PubMed PMID: WOS: 000309737900020. English.
42. Sofronic-Milosavljevic LJ, Radovic I, Ilic N, Majstorovic I, Cvetkovic J, Gruden-Movsesijan A. Application of dendritic cells stimulated with Trichinella spiralis excretory-secretory antigens alleviates experimental autoimmune encephalomyelitis. Med Microbiol Immunol. 2013;202(3):239–49.

43. Zhu B, Trikudanathan S, Zozulya AL, Sandoval-Garcia C, Kennedy JK, Atochina O, et al. Immune modulation by Lacto-N-fucopentaose III in experimental autoimmune encephalomyelitis. Clin Immunol. 2012;142(3):351–61. Pubmed Central PMCID: 3288504.
44. Wu Z, Nagano I, Asano K, Takahashi Y. Infection of non-encapsulated species of Trichinella ameliorates experimental autoimmune encephalomyelitis involving suppression of Th17 and Th1 response. Parasitol Res. 2010;107(5):1173–88.
45. Walsh KP, Brady MT, Finlay CM, Boon L, Mills KH. Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses. J Immunol. 2009;183(3):1577–86. PubMed PMID: WOS: 000268519500013. English.
46. Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K, et al. Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol. 2010;40(6):1682–96. Pubmed Central PMCID: 3179601.
47. Reyes JL, Espinoza-Jimenez AF, Gonzalez MI, Verdin L, Terrazas LI. Taenia crassiceps infection abrogates experimental autoimmune encephalomyelitis. Cell Immunol. 2011;267(2):77–87.
48. Gruden-Movsesijan A, Ilic N, Mostarica-Stojkovic M, Stosic-Grujicic S, Milic M, Sofronic-Milosavljevic L. Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic Trichinella spiralis infection in Dark Agouti rats. Parasite Immunol. 2010; 32(6):450–9.
49. Chiuso-Minicucci F, Van DB, Zorzella-Pezavento SF, Peres RS, Ishikawa LL, Rosa LC, et al. Experimental autoimmune encephalomyelitis evolution was not modified by multiple infections with Strongyloides venezuelensis. Parasite Immunol. 2011;33(5):303–8.
50. Radovic I, Gruden-Movsesijan A, Ilic N, Cvetkovic J, Mojsilovic S, Devic M, et al. Immunomodulatory effects of Trichinella spiralis-derived excretory-secretory antigens. Immunol Res. 2015;61(3):312–25.
51. Tundup S, Srivastava L, Harn Jr. DA. Polarization of host immune responses by helminth-expressed glycans. Ann N Y Acad Sci. 2012;1253:E1–13.
52. Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med. 2005;202(9):1199–212. Pubmed Central PMCID: 2213237.
53. Taylor MD, van der Werf N, Harris A, Graham AL, Bain O, Allen JE, et al. Early recruitment of natural CD4+ Foxp3+ Treg cells by infective larvae determines the outcome of filarial infection. Eur J Immunol. 2009;39(1):192–206.
54. Tadokoro CE, Vallochi AL, Rios LS, Martins GA, Schlesinger D, Mosca T, et al. Experimental autoimmune encephalomyelitis can be prevented and cured by infection with Trypanosoma cruzi. J Autoimmun. 2004;23(2):103–15. PubMed PMID: WOS: 000223912200002. English.
55. Wallberg M, Harris RA. Co-infection with Trypanosoma brucei brucei prevents experimental autoimmune encephalomyelitis in DBA/1 mice through induction of suppressor APCs. Int Immunol. 2005;17(6):721–8. PubMed PMID: WOS: 000230346000007. English.
56. Gong D, Shi W, Yi SJ, Chen H, Groffen J, Heisterkamp N. TGFbeta signaling plays a critical role in promoting alternative macrophage activation. BMC Immunol. 2012;13:31. Pubmed Central PMCID: 3406960.
57. Maizels RM, Pearce EJ, Artis D, Yazdanbakhsh M, Wynn TA. Regulation of pathogenesis and immunity in helminth infections. J Exp Med. 2009;206(10):2059–66. Pubmed Central PMCID: 2757871.
58. Wan YY, Flavell RA. TGF-beta and regulatory T cell in immunity and autoimmunity. J Clin Immunol. 2008;28(6):647–59. Pubmed Central PMCID: 2837280.
59. Beer RJ. The relationship between Trichuris trichiura (Linnaeus 1758) of man and Trichuris suis (Schrank 1788) of the pig. Res Vet Sci. 1976;20(1):47–54.
60. Navarro S, Ferreira I, Loukas A. The hookworm pharmacopoeia for inflammatory diseases. Int J Parasitol. 2013;43(3–4):225–31. PubMed PMID: WOS: 000315975700005. English.

61. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. N Engl J Med. 2004;351(8):799–807.
62. Blount D, Hooi D, Feary J, Venn A, Telford G, Brown A, et al. Immunologic profiles of persons recruited for a randomized, placebo-controlled clinical trial of hookworm infection. Am J Trop Med Hyg. 2009;81(5):911–6.
63. Pritchard DI, Brown A. Is Necator americanus approaching a mutualistic symbiotic relationship with humans? Trends Parasitol. 2001;17(4):169–72. PubMed PMID: WOS: 000169933900011. English.
64. Quinnell RJ, Bethony J, Pritchard DI. The immunoepidemiology of human hookworm infection. Parasite Immunol. 2004;26(11–12):443–54. PubMed PMID: WOS: 000227673900003. English.
65. Geiger SM, Caldas IR, Mc Glone BE, Campi-Azevedo AC, De Oliveira LM, Brooker S, et al. Stage-specific immune responses in human Necator americanus infection. Parasite Immunol. 2007;29(7):347–58. PubMed PMID: WOS: 000247855700003. English.
66. Palmer ED. Course of egg output over a 15 year period in a case of experimentally induced necatoriasis americanus, in the absence of hyperinfection. Am J Trop Med Hyg. 1955;4(4):756–7. PubMed PMID: WOS: A1955XG09400014. English.
67. Feary J, Venn A, Brown A, Hooi D, Falcone FH, Mortimer K, et al. Safety of hookworm infection in individuals with measurable airway responsiveness: a randomized placebo-controlled feasibility study. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2009;39(7):1060–8. Pubmed Central PMCID: 2728895.
68. Mortimer K, Brown A, Feary J, Jagger C, Lewis S, Antoniak M, et al. Dose-ranging study for trials of therapeutic infection with Necator americanus in humans. Am J Trop Med Hyg. 2006;75(5):914–20. PubMed PMID: WOS: 000242189100025. English.
69. Maxwell C, Hussain R, Nutman TB, Poindexter RW, Little MD, Schad GA, et al. The clinical and immunologic responses of normal human volunteers to low dose hookworm (Necator americanus) infection. Am J Trop Med Hyg. 1987;37(1):126–34.
70. Falcone FH, Pritchard DI. Parasite role reversal: worms on trial. Trends Parasitol. 2005;21(4):157–60. PubMed PMID: WOS: 000228286500003. English.
71. Pritchard DI, Blount DG, Schmid-Grendelmeier P, Till SJ. Parasitic worm therapy for allergy: is this incongruous or avant-garde medicine? Clin Exp Allergy. 2012;42(4):505–12. PubMed PMID: WOS: 000301532800005. English.
72. Feary JR, Venn AJ, Mortimer K, Brown AP, Hooi D, Falcone FH, et al. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy. 2010;40(2):299–306. PubMed PMID: WOS: 000273547500015. English.
73. Croese J, O'Neil J, Masson J, Cooke S, Melrose W, Pritchard D, et al. A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. Gut. 2006;55(1):136–7. Pubmed Central PMCID: 1856386.
74. Daveson AJ, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A, et al. Effect of hookworm infection on wheat challenge in celiac disease–a randomised double-blinded placebo controlled trial. Plos One. 2011;6(3):e17366. Pubmed Central PMCID: 3050888.
75. Fleming JO, Isaak A, Lee JE, Luzzio CC, Carrithers MD, Cook TD, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. Mult Scler. 2011;17(6):743–54. Pubmed Central PMCID: 3894910.
76. Bager P, Kapel C, Roepstorff A, Thamsborg S, Arnved J, Ronborg S, et al. Symptoms after ingestion of pig whipworm Trichuris suis eggs in a randomized placebo-controlled double-blind clinical trial. Plos One. 2011;6(8):e22346. PubMed PMID: WOS: 000293511900005. English.
77. http://clinicaltrials.gov/show/NCT00645749/msif-atlas-of-ms-2013-report.pdf.
78. Moynagh PN. The roles of Pellino E3 ubiquitin ligases in immunity. Nat Rev Immunol. 2014;14(2):122–31. PubMed PMID: WOS: 000331022400014. English.
79. Fleming JHL, Maksimovic J, Nace S, Luzzio C, Koehn M, Ritter A, Risa T, Lawler B, Maser A, Mundt P, Rolak L, Cook T, Field A, Fabry Z. Clinical Trial of Helminth-induced Immunomodulatory Therapy (HINT 2) in relapsing-remitting multiple sclerosis AAN annual meeting. Philadelphia: Neurology; 2014. p. P3.149.

80. Benzel F, Erdur H, Kohler S, Frentsch M, Thiel A, Harms L, et al. Immune monitoring of Trichuris suis egg therapy in multiple sclerosis patients. J Helminthol. 2012;86(3):339–47.
81. Lammie PJ, Katz SP. Immunoregulation in experimental filariasis. II. Responses to parasite and nonparasite antigens in jirds with Brugia pahangi. J Immunol. 1983;130(3):1386–9.
82. Graham SP, Trees AJ, Collins RA, Moore DM, Guy FM, Taylor MJ, et al. Down-regulated lymphoproliferation coincides with parasite maturation and with the collapse of both gamma interferon and interleukin-4 responses in a bovine model of onchocerciasis. Infect Immun. 2001;69(7):4313–9. Pubmed Central PMCID: 98501.
83. Rosche B, Werner J, Benzel FJ, Harms L, Danker-Hopfe H, Hellweg R. Serum levels of brain-derived neurotrophic factor (BNDF) in multiple sclerosis patients with Trichuris suis ova therapy. Parasite. 2013;20:55. Pubmed Central PMCID: 3866952. Niveaux seriques du facteur neurotrophique derive du cerveau (BNDF) chez des patients atteints de sclerose en plaques sous therapie par les oeufs de Trichuris suis.
84. Rosche B, Wernecke KD, Ohlraun S, Dorr JM, Paul F. Trichuris suis ova in relapsing-remitting multiple sclerosis and clinically isolated syndrome (TRIOMS): study protocol for a randomized controlled trial. Trials. 2013;14:112. Pubmed Central PMCID: 3680966.
85. Voldsgaard A, Bager P, Kapel C, Roepstorff A, Thamsborg S, Soendergaard H, et al. Trichuris suis ova therapy for relapsing multiple sclerosis – a safety study. Neurology. 2012;78:S30.005. PubMed PMID: WOS: 000303204802022. English.
86. http://clinicaltrials.gov/show/NCT01470521/msif-atlas-of-ms-2013-report.pdf.
87. Rook GA, Lowry CA, Raison CL. Hygiene and other early childhood influences on the subsequent function of the immune system. Brain Res. 2015;1617:47–62. doi: 10.1016/j.brainres.2014.04.004.
88. Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol. 2002;2(2):85–95.
89. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M. Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. Lancet Infect Dis. 2014;14(11):1150–62.

Chapter 12
Neurological Complications of Anti-TNF Treatments and Other Neurological Aspects of Inflammatory Bowel Disease

Su-Yin Lim and Cris S. Constantinescu

Abstract Inflammatory bowel diseases (IBD) result from dysregulated immune responses in the bowel. They are characterised by pathology mediated by immune cells with upregulated inflammatory profile. These disorders of immune regulation often coexist with other inflammatory conditions with altered immunoregulatory activities, including multiple sclerosis, rheumatoid arthritis, psoriasis, etc. Treatments targeting the pro-inflammatory cytokine, tumour necrosis factor alpha (TNFα), such as antibodies or soluble receptors, have revolutionised the management of IBD. However, paradoxically, such treatments have been associated with a risk of developing demyelinating disease, often typical multiple sclerosis. This chapter reviews the literature on the known prevalence and risk of demyelination in patients with IBD receiving TNF inhibitors, discusses potential mechanisms and also addresses the immunopathogenic, environmental and genetic commonalities of IBD and central nervous system demyelinating disease.

Keywords Autoimmunity • Biological • Comorbidity • Demyelination • Inflammatory bowel disease • Multiple sclerosis • TNF

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Introduction: Pathological Basis of TNFα in Inflammatory Bowel Disease and Multiple Sclerosis

The two main forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), share characteristic features of chronic, relapsing inflammation of the gastrointestinal tract, although they demonstrate distinct clinical and pathological features. CD most commonly affects the small intestine and colon, although any part of the GI tract can be affected. It is characterised by discontinuous or 'skip' ulcerated lesions and transmural inflammation. UC involves the colonic mucosa, usually extending to the rectum, characterised by mucosal inflammation, ulcers and crypt abscesses [1].

The exact aetiology of IBD is unknown. The development of IBD is believed to be, in part, due to genetic susceptibility and a dysregulated T-cell-mediated immunological response to enteric bacteria, along with other environmental factors [2]. TNFα has been identified as a crucial mediator in the inflammatory response in IBD, and TNF-inhibitory therapies have been effective in the treatment of both CD and UC where conventional therapy has failed [3].

TNFα is part of a large family of pleiotropic cytokines that induce signalling via two receptors, i.e. TNFRI and TNFRII. Cellular proliferation, survival, differentiation and death are mediated via complex signalling pathways, giving rise to inflammatory and immunomodulatory processes. Dysregulation of TNFα expression and of its signalling have been implicated in the pathogenesis of a variety of disorders including cancer, sepsis and autoimmune-mediated inflammatory disorders such as IBD, multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis and psoriatic arthritis (PsA). TNFα is secreted by macrophages, monocytes, lymphocytes, natural killer cells, microglia, fibroblasts, astrocytes and other immune and nonimmune cells. TNFα is expressed in response to a number of stimuli including infective antigens, tumour cells and a variety of complement and cytokines [4]. Signalling via TNFRI can induce both pro- and anti-apoptotic mechanisms via separate pathways [5], whilst activation of TNFRII is thought to enhance the actions of TNFRI and is involved in remyelination [6] and other pro-inflammatory effects [7, 8].

TNF inhibition (TNFi) is achieved by blocking TNFα activation of their receptors using a monoclonal antibody or soluble receptor. A number of TNFi agents are licensed for use in IBD – infliximab (Remicade, Merck Sharp & Dohme), adalimumab (Humira, Abbott Laboratories), certolizumab pegol (Cimzia, UCB) and golimumab (Simponi, Merck Sharp & Dohme). Infliximab, adalimumab and golimumab are monoclonal antibodies to TNFα, and certolizumab pegol is a PEGylated anti-TNFα antibody fragment.

Infliximab, adalimumab and certolizumab pegol have licensed indications in CD, whereas infliximab, adalimumab and golimumab are licensed in UC. Pivotal studies of infliximab, a chimeric monoclonal antibody to TNFα, demonstrated therapeutic efficacy in inducing and maintaining disease remission in luminal and fistulising CD [9–13] and in UC [14] compared to placebo. Adalimumab, a human monoclonal antibody, has shown comparable efficacy to infliximab in the induction of disease remission and

maintenance of remission in both CD [15–17] and UC [18], including those previously unresponsive to infliximab [19]. The newer agents, certolizumab pegol and golimumab, have both shown effectiveness in inducing and maintaining remission in CD [20] and UC [21], respectively. TNFi therapy reduces hospitalisations and surgical intervention in these patients [22]. Infliximab and adalimumab are currently recommended in the UK by the National Institute for Health and Care Excellence (NICE) for patients with severe, active CD who have not responded to or are intolerant of conventional therapy including immunosuppressive and/or corticosteroid treatment. Infliximab, adalimumab and golimumab are recommended by NICE for the treatment of moderate to severe UC in those who have failed or are unsuitable for conventional treatment.

MS is an immune-mediated inflammatory demyelinating disorder of the CNS, with a UK population prevalence of around 203 per 100,000 [23]. The disease is characterised by the appearance of demyelinating plaques disseminated in time and space. The exact aetiology of MS is uncertain and likely involves an interplay between genetic and environmental factors [24]. The immunopathogenesis of MS is shown to be mediated by autoreactive CD4+ T-cells, CD8+ T-cells, antibodies and components of the innate immune system [25].

MS and TNFα

TNFα is detected in MS lesions [26] and cerebrospinal fluid (CSF) of MS patients [27], showing a correlation with disease progression [28] and relapses [29]. TNFα contributes to oligodendrocyte damage and demyelination by signalling apoptotic pathways [30, 31], enhancing leukocyte transgression into the CNS via upregulation of endothelium-based cellular adhesion molecules at the blood–brain barrier [32] and promoting the expression of MHC I and II on neurons and glial cells which are targeted by MHC-restricted cytotoxic T-cells [33, 34]. In the animal model of MS, experimental autoimmune encephalomyelitis (EAE) administration of TNFα causes disease worsening [35]. Mice genetically engineered to overexpress TNFα developed inflammatory demyelination [36], whilst antagonism of TNFα prevented the onset of EAE [37–39].

Despite the role of TNFα in the pathogenesis of MS and its animal model, TNFα inhibition has been shown to worsen disease activity in MS patients. In an early phase I open-label study, infliximab was administrated to two MS patients with rapidly progressive disease [40]. Both patients showed an increase in contrast-enhancing MRI lesions and a corresponding rise in CSF leukocyte counts and IgG index, denoting increased disease activity. Subsequently, two MS patients with active disease were treated with lenercept, a soluble recombinant TNF receptor fusion protein, in a phase II double-blinded trial [41]. Lenercept had previously demonstrated efficacy in preventing the onset of demyelination in the animal model of MS [42]. The lenercept-treated patients showed a higher relapse rate and more severe clinical relapses compared to the placebo-treated group, leading to the cessation of TNFi use for treatment of MS.

Demyelination Associated with TNFi Therapy

There are an increasing number of reports from trial safety data, postmarketing surveillance and cases in the medical literature of the development of CNS demyelination with TNFi therapy for conditions such as IBD, RA, ankylosing spondylitis (AS), psoriasis, systemic lupus erythematosus (SLE) and vasculitis [43–58].

Deepak et al. [59] evaluated neurological adverse events with TNFi treatment (infliximab, adalimumab, certolizumab pegol and etanercept) reported to the FDA Adverse Event Reporting System (FAERS) between 1st January 2000 and 31st December 2009. 18.1 % of patients had IBD whilst the majority of patients had underlying RA (50.9 %). The most commonly reported neurological adverse event was peripheral neuropathy (38.3 %). Of the peripheral neuropathy cases, 16.6 % are composed of acute inflammatory demyelinating polyneuropathy (AIDP) and 5.1 % were chronic inflammatory demyelinating polyneuropathy (CIDP), whilst the remainder consisted of unclassified peripheral neuropathies, sensory neuropathies, motor neuropathies and sensorimotor neuropathies. CNS demyelination was the second most commonly reported neurological adverse event (19.8 %), in addition to reports of optic neuritis (ON) (13.6 %), transverse myelitis (3.4 %) and 'demyelination' where the site of disease was unspecified (3.4 %). Taking into account the temporal association, relevant reported past adverse events and plausible alternative causative factors, the majority of cases were scored as a 'possible' adverse event (71.4 %), whilst the rest were 'probable'. None met the criteria for 'definite' adverse events.

A number of cohort studies have attempted to determine whether TNFi therapy increases the risk of demyelination in IBD patients [50, 60–63]. The largest and most recent study [62] retrospectively examined a total of 9095 IBD patients (4342 with CD and 4753 with UC) across a region of North America. Median follow-up was 10.5 years. 5 of 3425 (0.15 %) patients who were exposed to TNFi treatment developed a CNS demyelinating disorder confirmed on the basis of clinical, MRI and laboratory assessment. Of these, four had an underlying diagnosis of CD and one had UC. Three of the patients with demyelination developed neurological symptoms during treatment with TNFi, one patient developed asymptomatic demyelinating lesions on MR imaging during treatment, and one patient with a past exposure to TNFi experienced progressive neurological symptoms whilst on azathioprine. Neurological symptoms either improved or resolved in two patients following discontinuation of TNFi, whereas a third patient went on to develop a relapsing–remitting demyelinating disease course. In the unexposed patients, 29 of 5670 were diagnosed with CNS demyelination, ten of which developed the condition after the onset of IBD (0.18 %). In this group, five had CD and five had UC. The relative risk of CNS demyelination with TNFi exposure in this study was 0.83 for IBD (95 % CI 0.28–2.42), 0.89 for CD (95 % CI 0.24–3.31) and 0.49 for UC (95 % CI 0.06–4.22), none of which showed statistical significance. The authors concluded that IBD patients exposed to TNFi did not appear to have a significantly increased risk of CNS demyelination over those who were not exposed to TNFi.

A retrospective cohort study by Andersen et al. [43] compared the risk of demyelinating disorders in 651 Danish IBD patients who were TNFi-treated between 1999 and 2005 to that of the general IBD population, with data gathered from four unrelated retrospective cohort studies of demyelinating diseases in IBD patients [64–67]. From the Danish cohort, four patients treated with TNFi agents were reported to develop neurological symptoms; however, only one was confirmed to have a demyelinating disorder in this case, MS – following investigations. They reported a standardised morbidity ratio of 4.2 for developing MS in the study cohort of TNFi-treated IBD patients, which was comparable to that of the general IBD population, leading the group to conclude that TNFi did not appear to significantly increase the risk of demyelination in this cohort.

In a more recent database study, Katsanos et al. [68] retrospectively reviewed cases of demyelination in patients with IBD including those who were treated with TNFi therapy, identified via MEDLINE and EMBASE. The study included 34 case reports, three case control studies [69–71] and eight cohort studies [50, 60, 61, 63–66] including one prospective study [72]. Cases of CNS demyelination are composed of MS or MS-like syndromes, acute disseminated encephalomyelitis (ADEM) and ON and demyelinating polyneuropathies such as Guillain-Barré syndrome (GBS), CIDP and MMN. The group found comparable and overlapping prevalence rates of demyelinating adverse events in TNFi-treated IBD patients compared to those who were treated with conventional therapy [mean prevalence of 0.65 % (0.2–2.5 %) and 0.48 % (range 0.41–1.2 %), respectively].

Other retrospective database studies of TNFi and demyelination in other chronic inflammatory conditions appear to support these findings. A study on the incidence of demyelination in patients with rheumatic diseases such as RA, AS and PsA treated with TNFi examined data from three pharmacovigilance sources – the Spanish registry of biological therapies in rheumatic diseases (BIOBADASER), the Spanish Pharmacovigilance Database of Adverse Drug Reactions (FEDRA) and major biomedical databases (PubMed, EMBASE and the Cochrane Library) [73]. Of 9256 patients receiving TNFi therapy for a total 21,425 patient-years, 14 patients were reported to have developed a demyelinating disease in the BIOBADASER database, including one case of MS, four of ON and one GBS. The incidence rate of demyelinating disease in this group of patients was estimated at 0.65 per 1000 patient-years (95 % CI 0.39–1.1). Nineteen cases of demyelination were reported in the FEDRA database, with some overlap of cases with BIOBADASER, of which nine had MS and seven had ON. 48 case reports from the major biomedical databases were reviewed, including ten cases of MS and 13 of ON. The authors concluded that the number of demyelination cases reported in the registry did not exceed that of the expected rate in the Spanish general population, and thus a direct link between TNFi and demyelination remains to be established.

From published care reports in the literature, the timing of onset of neurological symptoms from TNFi exposure appears wide ranging and does not always support a clear-cut temporal relationship. Reports of symptom onset varying from a few hours to up to 4 years from TNFi exposure have been described in IBD patients [68], whereas an unrelated case review of patients with inflammatory arthritides

report an interval of between 1 week and 5 months [74]. Demyelination has also been reported in patients on other forms of immunomodulation and DMARDs in the treatment of IBD, such as methotrexate, azathioprine and mercaptopurine [49, 75, 76]. Discontinuation of the TNFi tended to cause improvement or lead to resolution of neurological symptoms [47, 52, 58], whilst re-exposure to TNFi has been associated with symptom recurrence [62, 74]. However, this does not necessarily confirm an association; as MS typically runs a relapsing–remitting clinical course, acute demyelinating events often spontaneously improve or recover without specific treatment, and symptom change may reflect a response to steroid initiation or taper.

There is a lack of a clear explanation for the discordant effect of TNF inhibition in conditions such as IBD and RA compared to MS. A possible hypothesis relates to the lack of penetration by TNFi agents into the CNS via the blood–brain barrier [77] and subsequent failure of TNFα antagonism to take place locally, in contrast to the joints and bowel. In the aforementioned phase I study of infliximab in MS, the monoclonal antibody was not detected in the CSF of patients despite the presence of blood–brain barrier disruption [40]. Another theory is based on the dissemination of a latent or recently acquired infection by TNFi agents, inducing an autoimmune response [78]. A further explanation for the discordant effect of TNFi lies in the heterogeneity of the cytokine TNFα and its disparate effects. TNFα promotes remyelination and oligodendrocyte regeneration via activation of the TNFRII receptor [6], and therefore blockade of this process can cause MS worsening. TNFα-mediated apoptosis is important for the deletion of autoreactive cytotoxic T-cells, thus playing a key role in the regulation of autoimmunity [79]. Selective TNFi inhibition, particularly of the TNFRI receptor or its function, may be key to inhibiting the pro-inflammatory properties of TNFα whilst sparing its neuroprotective and immunoregulatory effects [80].

Imaging Studies in TNFi-Treated Patients

It remains unclear whether TNFi treatment unmasked pre-existing (subclinical) MS or whether it induced the onset of new demyelination in these patients. A contributing factor to this ongoing uncertainty is the lack of prospective imaging studies in this field. An early study by van der Bilj [81] examined quantitative MRI metrics (magnetisation transfer ratio [MTR], apparent diffusion coefficient and spectroscopy) before and up to 7 days after treatment with TNFi in seven patients with inflammatory arthritis (five rheumatoid arthritis and two psoriatic arthritis). Reduction in MTR correlates with demyelination, axonal loss and other inflammatory changes in MS [82]. Results showed a significant decrease in the white and grey matter MTR histogram peak height following treatment, although there were no significant changes to the other MRI metrics. The findings would support a possible diffuse CNS inflammatory process related to TNFi treatment; however, without longer term follow-up, it was not possible to ascertain if the MTR changes were transient or clinically relevant in this study.

Our own subsequent imaging study (Lim SY, et al; manuscript in preparation) quantitatively examined white matter lesions and the normal-appearing white matter (NAWM) in a cohort of 15 patients who were receiving TNFi treatment for either RA or AS and compared the findings with 11 healthy controls and seven RRMS controls, obtaining MTR and T1 relaxation times (T1RT). Like MTR, the latter has been shown to correlate with demyelination and axonal loss in MS [82]. Incidental white matter lesions were a common finding in the RA and AS patients, as well as the healthy controls. We demonstrated that white matter lesions and the normal-appearing white matter MTR and T1RT of TNFi-treated patients did not differ to that of healthy controls whilst being significantly abnormal in the RRMS patients. The findings do not support the presence of clinically asymptomatic CNS demyelination in our treatment cohort.

A recent study by Kaltsonoudis [83] prospectively followed 75 patients with RA and spondyloarthropathies treated with TNFi therapy for a mean study period of 18 months. Neurological assessment, MRI and neurophysiological testing were performed in all the patients prior to commencement of TNFi treatment. A total of 38 patients were treated with infliximab, 19 with adalimumab and 18 with etanercept. A proportion of patients remained on concomitant steroids and/or other immunomodulatory agents. Three patients reportedly developed neurological complications – the first patient developed CNS demyelination with corresponding periventricular white matter lesions, a peripheral facial nerve palsy and peroneal mononeuropathy. The second patient developed a unilateral optic neuritis, whilst the third developed a sensory-predominant peripheral neuropathy. Symptom onset ranged from 6 to 25 months from initiation of therapy. In all three cases, TNFi therapy was discontinued. The second and third patients experienced a re-emergence of symptoms shortly following re-initiation of treatment, and TNFi was permanently discontinued thereafter. Incidentally, the study also identified two patients with asymptomatic white matter lesions on MR imaging, described as a radiologically isolated syndrome, prior to commencement of TNFi therapy. This highlighted an important consideration of performing baseline imaging in those being considered for TNFi treatment.

Association Between TNFi and Other Immune Disorders

TNFi therapy has also been associated with the development of other immune disorders. The induction of clinically asymptomatic ANA and anti-dsDNA autoantibodies have been observed in 53 and 35 % of CD patients treated with infliximab, respectively [84], and cases of infliximab-induced lupus have been described [61]. Induction of anti-cardiolipin antibodies has been associated with TNFi therapy [85] which may be associated with clinical features of antiphospholipid syndrome in a significant proportion of patients [86]. Vasculitis and, in particular, cutaneous vasculitis have also been reported in association with TNFi agents [87, 88]. Other autoimmune inflammatory disorders reported in association with TNFi treatment include sarcoidosis, autoimmune hepatitis, psoriasis and myositis, amongst others [61, 87, 88]. However, like the reported associations between TNFi and demyelinating disorders,

a cause and effect association between TNFi and paradoxical induction of autoimmune diseases cannot be made conclusively, given the overall lack of controlled studies and the presence of underlying autoimmune spectrum disorders for which these patients are receiving TNFi therapy in the first place.

Association Between MS and IBD

The concomitance of MS and other immune-mediated disorders has long been recognised [69, 70, 89–97] including the finding of a higher incidence of MS amongst IBD patients prior to the use of TNFi agents [64–67, 98, 99].

IBD in MS

A prospective study [100] of 658 MS patients showed significantly increased rates of IBD, with five patients identified as having UC (0.8 %) and two with CD (0.3 %). The odds ratio for UC and CD in the MS cohort were found to be 3.15 (91 % CI 7.64–1.30) and 3.17 (95 % CI 9.95–1.01), respectively. The study also demonstrated significantly increased rates of asthma, type 1 diabetes, pernicious anaemia, autoimmune thyroid disease, uveitis and seronegative spondyloarthropathies compared to the general population. A North American study of over 5000 MS patients reported the prevalence of IBD in this cohort of 0.79 % and an odds ratio of 1.7 (95 % CI 1.2–2.5) compared to the control population [69]. The study also reported an increased risk of other immune-mediated conditions in MS patients, including uveitis, Guillain-Barre syndrome and bullous pemphigoid.

A recent systematic review and meta-analysis of autoimmune diseases in MS patients and their families [101] derived a significant odds ratio of 1.37 (95 % CI 1.12–1.69) for CD in patients with MS based on four population-based studies [100, 102–104]. They also derived an odds ratio 2.26 for UC in MS (95 % CI 1.23–4.14) from six population-base studies [76, 100, 102–105]. The overall odds ratio for IBD in MS was 1.56 (95 % CI 1.28–1.90). The relatives of patients with MS however did not appear at significant risk of IBD.

MS in IBD

A retrospective cohort study [65] of 7988 CD cases and 12185 UC cases obtained from the UK-based General Practice Research Database demonstrated a higher incidence of demyelinating disease (optic neuritis, demyelination and/or MS) in CD and UC compared to matched controls, although only the UC group showed statistical significance (incidence rate ratio 2.63; 95 % CI 1.29–5.15). The group also

conducted a cross-sectional study in the same cohort of patients, finding a significantly higher prevalence of CD (OR 1.54; 95 % CI 1.03–2.32) and UC (OR 1.75; 95 % CI 1.28–2.39) in comparison to matched controls. Echoing the findings from the retrospective cohort study above, Bernstein et al. [64] also found a significantly increased likelihood of MS in UC patients compared to controls (prevalence rate of at least 1.81; 95 % CI 1.35–2.42) amongst a cohort of 8072 IBD sufferers in Canada. In another study from North America, Kimura et al. [66] found a prevalence of MS amongst 474 newly diagnosed IBD patients between 1950 and 1995 at 3.7 times higher than that expected for the population.

The nature of the association between IBD and peripheral neuropathy, particularly demyelinating neuropathy, is less clear, probably reflecting the variations in the clinical definition of neuropathy and its wide-ranging aetiology. In a prospective study of 31 CD and 51 UC patients followed up for a period of 1 year [72], at least 13.4 % of patients were diagnosed with a cryptogenic large-fibre or small-fibre neuropathy based on clinical and/or neurophysiological assessment, including a single case of demyelinating neuropathy at the diagnosis of CD, leading the authors to conclude that IBD may be a cause of neuropathy either due to immune mechanisms or possible undiagnosed nutritional deficiencies. On the other hand, Bernstein et al. [64] reported a relatively low prevalence of peripheral neuropathy in IBD of 0.10 % in CD and 0.18 % in UC. A subsequent retrospective cohort study of 772 IBD patients [106] extending from 1940 to 2004 identified only nine cases of peripheral neuropathy, comprising either a chronic large-fibre sensory-predominant polyneuropathy or an immune radiculoplexus neuropathy, giving a relatively low cumulative incidence of 2.4 % over 30 years.

The observed co-occurrence of IBD and MS/demyelination in patients pre-dating the use of biological therapy has led to putative genetic and immunopathological associations between the two conditions. Both MS and IBD affect relatively young populations [107, 108]. They share a similar pathogenesis of dysregulated T-helper 1 (Th1) cell function, and recent studies have implicated Th17 cells, a subset of helper T-cells, in the pathogenesis of autoimmune inflammatory diseases such as MS and IBD [109]. The incidence of IBD appears to be highest amongst populations with highest rates of MS [107] and, like MS, follow a latitudinal pattern of geographical distribution [110]. Genomic variations in the major histocompatibility complex (MHC) region are known to confer susceptibility to or protection from autoimmunity including MS and IBD [111, 112]. Genome-wide association studies have identified a number of other shared gene loci conferring susceptibility to IBD as well as MS, for example, the IL2RA (alpha-subunit of IL-2 receptor) on chromosome 10p15 [113]. The hypothesis of genetic susceptibility to autoimmunity is further supported by a clustering study [90] which identified a higher than expected prevalence of IBD in patients with familial MS and their first degree relatives, noting a common variant of the CTLA4 (cytotoxic T-lymphocyte-antigen 4) gene in the families involved.

It is being increasingly postulated that the gut microflora plays an important role in the pathogenesis of systemic inflammatory diseases including MS and IBD [2, 114]. Alteration of gut flora via oral administration of antibiotics ameliorates inflammatory activity in the mouse model of MS, experimental autoimmune

encephalomyelitis (EAE) [115]. Similarly, microbe-directed treatments including faecal transplantation have shown potential in the treatment of IBD, although further study and clinical trials are warranted [116].

Vitamin D has been identified as an important modulator in the adaptive and innate immune response [117]. Vitamin D promotes T regulatory cell function [118] and regulates MHC class II gene expression [119] in MS, and its deficiency is regarded as an environmental risk factor in the development of MS [120]. Vitamin D stimulates the expression of the NOD2/CARD15/IBD1 gene, a susceptibility gene for CD [121], and its deficiency correlates with a higher risk of CD [122].

Extra-intestinal manifestations of IBD are commonly reported, potentially arising from intestinal dysfunction (e.g. nutritional deficiencies), complications from immunotherapy or other autoimmune diseases not directly linked to IBD, for example, systemic vasculitis, polymyositis and Sjogren's syndrome [123]. Neurological conditions reported in IBD patients include peripheral neuropathies, myelopathy, myopathy, myasthenia gravis and cerebrovascular disease [124, 125].

Conclusion

Despite a clear acknowledgement of the risk of demyelination with TNFi therapy, a causal association remains unconfirmed. IBD and MS share similar immunopathogenic characteristics, and the likelihood of co-occurrence may confound the risks associated with TNFi. The risk of CNS demyelination in IBD patients is estimated at over three times that of the general population [100]. However, the concerns about developing a demyelinating disorder with TNFi therapy are fully justified. It is essential that patients are counselled on the risk of demyelination prior to commencement of treatment with TNFi agents and that treatment is avoided in those with suspected or a known diagnosis or of MS.

References

1. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol. 2003;3(7):521–33.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427–34.
3. Nielsen OH, Ainsworth MA. Tumor necrosis factor inhibitors for inflammatory bowel disease. N Engl J Med. 2013;369(8):754–62.
4. Rossard TP. Tumor necrosis factor, vol. xii. New York: Nova Biomedical Books; 2009. p. 246.
5. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 2005;115(1):1–20.
6. Arnett HA, et al. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci. 2001;4(11):1116–22.
7. Tartaglia LA, Pennica D, Goeddel DV. Ligand passing: the 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem. 1993;268(25):18542–8.

8. Weiss T, et al. Enhancement of TNF receptor p60-mediated cytotoxicity by TNF receptor p80: requirement of the TNF receptor-associated factor-2 binding site. J Immunol. 1997;158(5):2398–404.
9. Hanauer SB, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359(9317):1541–9.
10. Present DH, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med. 1999;340(18):1398–405.
11. Rutgeerts P, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology. 1999;117(4):761–9.
12. Sands BE, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med. 2004;350(9):876–85.
13. Targan SR, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med. 1997;337(15):1029–35.
14. Rutgeerts P, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2005;353(23):2462–76.
15. Colombel JF, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gastroenterology. 2007;132(1):52–65.
16. Hanauer SB, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. Gastroenterology. 2006;130(2):323–33; quiz 591.
17. Sandborn WJ, et al. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56(9):1232–9.
18. Sandborn WJ, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2012;142(2):257–65.e1–3.
19. Afif W, et al. Open-label study of adalimumab in patients with ulcerative colitis including those with prior loss of response or intolerance to infliximab. Inflamm Bowel Dis. 2009;15(9):1302–7.
20. Schreiber S. Crohn's disease--infliximab, adalimumab and certolizumab-pegol: clinical value of anti-TNF-alpha treatment. Dtsch Med Wochenschr. 2007;132(34–35):1770–4.
21. Sandborn WJ, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology. 2014;146(1):96–109.e1.
22. Lichtenstein GR, et al. Infliximab maintenance treatment reduces hospitalizations, surgeries, and procedures in fistulizing Crohn's disease. Gastroenterology. 2005;128(4):862–9.
23. Mackenzie IS, et al. Incidence and prevalence of multiple sclerosis in the UK 1990–2010: a descriptive study in the General Practice Research Database. J Neurol Neurosurg Psychiatry. 2013;85(1):76–84.
24. Ebers GC. Environmental factors and multiple sclerosis. Lancet Neurol. 2008;7(3):268–77.
25. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol. 2005; 23:683–747.
26. Selmaj K, et al. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. J Clin Invest. 1991;87(3):949–54.
27. Hauser SL, et al. Cytokine accumulations in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. Neurology. 1990;40(11):1735–9.
28. Sharief MK, Hentges R. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N Engl J Med. 1991;325(7):467–72.
29. Beck J, et al. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand. 1988;78(4):318–23.
30. Selmaj K, et al. Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. J Immunol. 1991;147(5):1522–9.
31. Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol. 1988;23(4):339–46.

32. Carlos TM, et al. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. Blood. 1990;76(5):965–70.
33. Lavi E, et al. Tumor necrosis factor induces expression of MHC class I antigens on mouse astrocytes. J Neuroimmunol. 1988;18(3):245–53.
34. Benveniste EN, Sparacio SM, Bethea JR. Tumor necrosis factor-alpha enhances interferon-gamma-mediated class II antigen expression on astrocytes. J Neuroimmunol. 1989; 25(2–3):209–19.
35. Kuroda Y, Shimamoto Y. Human tumor necrosis factor-alpha augments experimental allergic encephalomyelitis in rats. J Neuroimmunol. 1991;34(2–3):159–64.
36. Probert L, et al. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. Proc Natl Acad Sci U S A. 1995;92(24):11294–8.
37. Baker D, et al. Control of established experimental allergic encephalomyelitis by inhibition of tumor necrosis factor (TNF) activity within the central nervous system using monoclonal antibodies and TNF receptor-immunoglobulin fusion proteins. Eur J Immunol. 1994; 24(9):2040–8.
38. Ruddle NH, et al. An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J Exp Med. 1990;172(4):1193–200.
39. Selmaj K, Raine CS, Cross AH. Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann Neurol. 1991;30(5):694–700.
40. van Oosten BW, et al. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. Neurology. 1996;47(6):1531–4.
41. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/ MRI Analysis Group. Neurology. 1999;53(3):457–65.
42. Klinkert WE, et al. TNF-alpha receptor fusion protein prevents experimental auto-immune encephalomyelitis and demyelination in Lewis rats: an overview. J Neuroimmunol. 1997;72(2):163–8.
43. Andersen NN, et al. Occurrence of demyelinating diseases after anti-TNFalpha treatment of inflammatory bowel disease: a Danish Crohn Colitis Database study. J Crohns Colitis. 2008;2(4):304–9.
44. Bouchra A, Benbouazza K, Hajjaj-Hassouni N. Guillain-Barre in a patient with ankylosing spondylitis secondary to ulcerative colitis on infliximab therapy. Clin Rheumatol. 2009;28 Suppl 1:S53–5.
45. Dubcenco E, et al. Neurological symptoms suggestive of demyelination in Crohn's disease after infliximab therapy. Eur J Gastroenterol Hepatol. 2006;18(5):565–6.
46. Enayati PJ, Papadakis KA. Association of anti-tumor necrosis factor therapy with the development of multiple sclerosis. J Clin Gastroenterol. 2005;39(4):303–6.
47. Felekis T, et al. Reversible bilateral optic neuritis after Infliximab discontinuation in a patient with Crohn's disease. J Crohns Colitis. 2009;3(3):212–4.
48. Freeman HJ, Flak B. Demyelination-like syndrome in Crohn's disease after infliximab therapy. Can J Gastroenterol. 2005;19(5):313–6.
49. Gondim FA, et al. Peripheral neuropathy in patients with inflammatory bowel disease. Brain. 2005;128(Pt 4):867–79.
50. Lees CW, et al. The safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. Aliment Pharmacol Ther. 2009;29(3):286–97.
51. Lozeron P, et al. Long-term course of demyelinating neuropathies occurring during tumor necrosis factor-alpha-blocker therapy. Arch Neurol. 2009;66(4):490–7.
52. Mejico LJ. Infliximab-associated retrobulbar optic neuritis. Arch Ophthalmol. 2004;122 (5):793–4.
53. Nozaki K, et al. Neurological deficits during treatment with tumor necrosis factor-alpha antagonists. Am J Med Sci. 2011;342(5):352–5.

54. Ohyagi M, et al. Chronic inflammatory demyelinating polyradiculoneuropathy in a patient with Crohn's disease. Intern Med. 2013;52(1):125–8.
55. Ouakaa-Kchaou A, et al. Retrobulbar optic neuritis associated with infliximab in a patient with Crohn's disease. J Crohns Colitis. 2009;3(2):131–3.
56. Shin IS, et al. Guillain-Barre and Miller Fisher syndromes occurring with tumor necrosis factor alpha antagonist therapy. Arthritis Rheum. 2006;54(5):1429–34.
57. Solomon AJ, et al. Inflammatory neurological disease in patients treated with tumor necrosis factor alpha inhibitors. Mult Scler. 2011;17(12):1472–87.
58. Weinshenker BG, Sandborn WJ. Demyelination during anti-tumor necrosis factor alpha therapy with infliximab for Crohn's disease. Inflamm Bowel Dis. 2004;10(1):28–31.
59. Deepak P, et al. Neurological events with tumour necrosis factor alpha inhibitors reported to the Food and Drug Administration Adverse Event Reporting System. Aliment Pharmacol Ther. 2013;38(4):388–96.
60. Caspersen S, et al. Infliximab for inflammatory bowel disease in Denmark 1999–2005: clinical outcome and follow-up evaluation of malignancy and mortality. Clin Gastroenterol Hepatol. 2008;6(11):1212–7; quiz 1176.
61. Colombel JF, et al. The safety profile of infliximab in patients with Crohn's disease: the Mayo clinic experience in 500 patients. Gastroenterology. 2004;126(1):19–31.
62. De Felice KM, et al. Idiopathic inflammatory demyelinating disease of the central nervous system in patients with inflammatory bowel disease: retrospective analysis of 9095 patients. Aliment Pharmacol Ther. 2015;41(1):99–107.
63. Hamzaoglu H, et al. Safety of infliximab in Crohn's disease: a large single-center experience. Inflamm Bowel Dis. 2010;16(12):2109–16.
64. Bernstein CN, Wajda A, Blanchard JF. The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. Gastroenterology. 2005;129(3):827–36.
65. Gupta G, Gelfand JM, Lewis JD. Increased risk for demyelinating diseases in patients with inflammatory bowel disease. Gastroenterology. 2005;129(3):819–26.
66. Kimura K, et al. Concurrence of inflammatory bowel disease and multiple sclerosis. Mayo Clin Proc. 2000;75(8):802–6.
67. Rang EH, Brooke BN, Hermon-Taylor J. Association of ulcerative colitis with multiple sclerosis. Lancet. 1982;2(8297):555.
68. Katsanos AH, Katsanos KH. Inflammatory bowel disease and demyelination: more than just a coincidence? Expert Rev Clin Immunol. 2014;10(3):363–73.
69. Langer-Gould A, et al. Autoimmune diseases prior to the diagnosis of multiple sclerosis: a population-based case–control study. Mult Scler. 2010;16(7):855–61.
70. Marrie RA, et al. A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: overview. Mult Scler. 2013;21(3):263–81.
71. Tremlett HL, et al. Asthma and multiple sclerosis: an inverse association in a case–control general practice population. QJM. 2002;95(11):753–6.
72. Oliveira GR, et al. Peripheral neuropathy and neurological disorders in an unselected Brazilian population-based cohort of IBD patients. Inflamm Bowel Dis. 2008;14(3):389–95.
73. Cruz Fernandez-Espartero M, et al. Demyelinating disease in patients treated with TNF antagonists in rheumatology: data from BIOBADASER, a pharmacovigilance database, and a systematic review. Semin Arthritis Rheum. 2011;41(3):524–33.
74. Mohan N, et al. Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides. Arthritis Rheum. 2001;44(12):2862–9.
75. Constantinescu CS, Gran B. Multiple sclerosis: autoimmune associations in multiple sclerosis. Nat Rev Neurol. 2010;6(11):591–2.
76. Pokorny CS, Beran RG, Pokorny MJ. Association between ulcerative colitis and multiple sclerosis. Intern Med J. 2007;37(10):721–4.
77. Robinson WH, Genovese MC, Moreland LW. Demyelinating and neurologic events reported in association with tumor necrosis factor alpha antagonism: by what mechanisms could tumor necrosis factor alpha antagonists improve rheumatoid arthritis but exacerbate multiple sclerosis? Arthritis Rheum. 2001;44(9):1977–83.

78. Prinz JC. Autoimmune-like syndromes during TNF blockade: does infection have a role? Nat Rev Rheumatol. 2011;7(7):429–34.
79. Cope AP. Regulation of autoimmunity by proinflammatory cytokines. Curr Opin Immunol. 1998;10(6):669–76.
80. Kassiotis G, Kollias G. Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: implications for pathogenesis and therapy of autoimmune demyelination. J Exp Med. 2001;193(4):427–34.
81. van der Bijl AE, et al. Advanced magnetic resonance imaging of the brain in patients treated with TNF-alpha blocking agents. Clin Exp Rheumatol. 2007;25(2):301–4.
82. Mottershead JP, et al. High field MRI correlates of myelin content and axonal density in multiple sclerosis–a post-mortem study of the spinal cord. J Neurol. 2003;250 (11):1293–301.
83. Kaltsonoudis E, et al. Neurological adverse events in patients receiving anti-TNF therapy: a prospective imaging and electrophysiological study. Arthritis Res Ther. 2014;16(3):R125.
84. Nancey S, et al. Infliximab treatment does not induce organ-specific or nonorgan-specific autoantibodies other than antinuclear and anti-double-stranded DNA autoantibodies in Crohn's disease. Inflamm Bowel Dis. 2005;11(11):986–91.
85. Jonsdottir T, et al. Treatment with tumour necrosis factor alpha antagonists in patients with rheumatoid arthritis induces anticardiolipin antibodies. Ann Rheum Dis. 2004;63 (9):1075–8.
86. Ramos-Casals M, et al. Autoimmune diseases induced by biological agents: a double-edged sword? Autoimmun Rev. 2010;9(3):188–93.
87. Miehsler W, et al. A decade of infliximab: the Austrian evidence based consensus on the safe use of infliximab in inflammatory bowel disease. J Crohns Colitis. 2010;4(3):221–56.
88. Ramos-Casals M, et al. Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. Medicine (Baltimore). 2007;86(4):242–51.
89. Baker HW, et al. Multiple sclerosis and autoimmune diseases. Aust N Z J Med. 1972;2(3):256–60.
90. Barcellos LF, et al. Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study. Lancet Neurol. 2006;5(11):924–31.
91. Broadley SA, et al. Autoimmune disease in first-degree relatives of patients with multiple sclerosis. A UK survey. Brain. 2000;123(Pt 6):1102–11.
92. de Lau LM, et al. Acute CNS white matter lesions in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2009;15(4):576–80.
93. Gotkine M, Fellig Y, Abramsky O. Occurrence of CNS demyelinating disease in patients with myasthenia gravis. Neurology. 2006;67(5):881–3.
94. Heinzlef O, et al. Autoimmune diseases in families of French patients with multiple sclerosis. Acta Neurol Scand. 2000;101(1):36–40.
95. Lo R, Feasby TE. Multiple sclerosis and autoimmune diseases. Neurology. 1983;33(1):97–8.
96. Midgard R, et al. Multiple sclerosis and chronic inflammatory diseases. A case–control study. Acta Neurol Scand. 1996;93(5):322–8.
97. Toussirot E, et al. Association of rheumatoid arthritis with multiple sclerosis: report of 14 cases and discussion of its significance. J Rheumatol. 2006;33(5):1027–8.
98. Minuk GY, Lewkonia RM. Possible familial association of multiple sclerosis and inflammatory bowel disease. N Engl J Med. 1986;314(9):586.
99. Sadovnick AD, Paty DW, Yannakoulias G. Concurrence of multiple sclerosis and inflammatory bowel disease. N Engl J Med. 1989;321(11):762–3.
100. Edwards LJ, Constantinescu CS. A prospective study of conditions associated with multiple sclerosis in a cohort of 658 consecutive outpatients attending a multiple sclerosis clinic. Mult Scler. 2004;10(5):575–81.
101. Dobson R, Giovannoni G. Autoimmune disease in people with multiple sclerosis and their relatives: a systematic review and meta-analysis. J Neurol. 2013;260(5):1272–85.

102. Nielsen NM, et al. Autoimmune diseases in patients with multiple sclerosis and their first-degree relatives: a nationwide cohort study in Denmark. Mult Scler. 2008;14(6):823–9.
103. Ramagopalan SV, et al. Autoimmune disease in families with multiple sclerosis: a population-based study. Lancet Neurol. 2007;6(7):604–10.
104. Roshanisefat H, et al. Shared genetic factors may not explain the raised risk of comorbid inflammatory diseases in multiple sclerosis. Mult Scler. 2012;18(10):1430–6.
105. De Keyser J. Autoimmunity in multiple sclerosis. Neurology. 1988;38(3):371–4.
106. Figueroa JJ, et al. Peripheral neuropathy incidence in inflammatory bowel disease: a population-based study. Neurology. 2013;80(18):1693–7.
107. Green C, et al. A population-based ecologic study of inflammatory bowel disease: searching for etiologic clues. Am J Epidemiol. 2006;164(7):615–23; discussion 624–8.
108. Ramagopalan SV, Sadovnick AD. Epidemiology of multiple sclerosis. Neurol Clin. 2011;29(2):207–17.
109. Maddur MS, et al. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol. 2012;181(1):8–18.
110. Khalili H, et al. Geographical variation and incidence of inflammatory bowel disease among US women. Gut. 2012;61(12):1686–92.
111. Fernando MM, et al. Defining the role of the MHC in autoimmunity: a review and pooled analysis. PLoS Genet. 2008;4(4), e1000024.
112. Lettre G, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. Hum Mol Genet. 2008;17(R2):R116–21.
113. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. Gastroenterology. 2011;140(6):1704–12.
114. Kamada N, et al. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013;13(5):321–35.
115. Ochoa-Reparaz J, et al. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol. 2009;183(10):6041–50.
116. Hansen JJ, Sartor RB. Therapeutic manipulation of the microbiome in IBD: current results and future approaches. Curr Treat Options Gastroenterol. 2015;13(1):105–20.
117. Aranow C. Vitamin D and the immune system. J Invest Med. 2011;59(6):881–6.
118. Smolders J, et al. Vitamin D status is positively correlated with regulatory T cell function in patients with multiple sclerosis. PLoS One. 2009;4(8), e6635.
119. Ramagopalan SV, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS Genet. 2009;5(2), e1000369.
120. Munger KL, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60–5.
121. Wang TT, et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. J Biol Chem. 2010;285(4):2227–31.
122. Ananthakrishnan AN, et al. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. Gastroenterology. 2012;142(3):482–9.
123. Danese S, et al. Extraintestinal manifestations in inflammatory bowel disease. World J Gastroenterol. 2005;11(46):7227–36.
124. Lossos A, et al. Neurologic aspects of inflammatory bowel disease. Neurology. 1995;45(3 Pt 1):416–21.
125. Moris G. Inflammatory bowel disease: an increased risk factor for neurologic complications. World J Gastroenterol. 2014;20(5):1228–37.

Chapter 13
Intestinal Bacterial Antigens, Toxin-Induced Pathogenesis and Immune Cross-Reactivity in Neuromyelitis Optica and Multiple Sclerosis

Cris S. Constantinescu and I-Jun Chou

Abstract Multiple sclerosis (MS) and neuromyelitis optica (NMO) are chronic, potentially disabling, inflammatory autoimmune demyelinating diseases of the central nervous system. Although they share clinical, pathological and immunological features, MS and NMO are now considered two separate entities, and there is evidence that their pathogenesis is different. The latter is now known to be mediated by antibodies against the water channel, aquaporin-4, associated with complement-mediated damage. Environmental factors have been implicated in the pathogenesis of both of these conditions. Among these, infectious factors seem to play a key role. One mechanism whereby infection triggers autoimmunity is molecular mimicry resulting in immune cross-reactivity between infectious antigens and autoantigens. Recently, a number of studies have pointed to an immunological cross-reactivity between intestinal bacteria and aquaporin-4, providing a potential pathophysiological mechanism for NMO. The bacteria involved were *Clostridium* and *E. coli*. The immune cross-reactivity is not restricted to antibodies but also involves T cells against aquaporin-4 that also recognises clostridium epitopes. Interestingly, *Clostridium perfringens* and its immunological or direct neurotoxic effects (e.g. disruption of the blood-brain barrier) have also been implicated in MS. This chapter reviews the relevant data regarding the role of these gut bacteria and the immune responses they trigger in MS and NMO with some insights into the pathogenesis of these inflammatory demyelinating diseases.

Keywords Neuromyelitis optica • Intestinal bacteria • *Clostridium* • *E. coli* • Multiple sclerosis • Molecular mimicry • Cross-reactivity • Blood-brain barrier • Toxin

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Introduction

Multiple sclerosis (MS) is an immune-mediated inflammatory demyelinating disease of the central nervous system (CNS) affecting an estimated 2.5 million people worldwide and more than 120,000 in the United Kingdom [1, 2]. MS is a major cause of long-term neurological disability in young people. The pathology of MS is characterised by inflammation, demyelination, axonal loss and gliosis in the CNS in a multifocal distribution. Clinically, it manifests as relapsing and remitting neurological deficits (relapsing remitting MS, RRMS), which often evolve subsequently into a gradual progressive deterioration (secondary progressive MS, SPMS). A minority of patients (~15 %) experience a gradual deterioration from the start (primary progressive MS, PPMS). The cause of MS is unknown, but it believed to be the result of a combination of multiple genetic susceptibility factors and environmental triggers [1].

Comprehensive large genetic studies including genome-wide association studies have identified more than 100 genes linked to MS [3]. These genes are virtually all involved in the immune response, underscoring the immune-mediated mechanisms. Although the functional contributions of these immune response genes to the aetiology of MS are only beginning to be explored [4, 5], the further development of immunotherapies for the disease, as well as the use of relevant experimental models, seems justified.

All elements of the immune system contribute to the pathogenesis of MS. Accordingly, autoreactive T cells, elements of the innate immune system and B cells are all implicated in a complex, dysregulated network. Immunoregulatory factors including regulatory T cells seem to be defective in MS.

The first attack and the subsequent relapses of MS often follow infections or global immune activation by vaccines. A number of infectious agents have been implicated as environmental risk factors for MS, and currently the one that appears most consistently associated with MS, based on immunological, epidemiological and virological evidence, is the human gamma herpesvirus, the Epstein-Barr virus [6, 7].

Neuromyelitis optica (NMO) is also a chronic inflammatory demyelinating disease of the CNS, affecting preferentially the optic nerves and the spinal cord [8]. Like MS, it can cause significant disability. In fact this is often more severe than that seen in MS. It can also have a relapsing and remitting or a chronic progressive course. NMO is much less frequent than MS and was considered for a long time a rare, more aggressive variant of MS. It was not until a decade ago that the discovery of the NMO autoantibodies [9], directed against the water channel aquaporin-4 (Aqp4) [10], and the evidence implicating these antibodies directly in NMO pathogenesis [11] provided proof of distinct immunopathogenic mechanisms in MS and NMO.

Despite the immunological complexity of MS, the prevailing concept is that T lymphocyte plays a central and decisive role [12]. Although therapies targeting B cells have shown promise in MS, the most important role of B cells in MS appears to be their function as antigen-presenting cells. In contrast, NMO is mediated primarily by antibodies which can fix complement, and this is the main pathogenesis

mechanism [8]. Indeed, NMO is frequently associated with other antibody-mediated autoimmune diseases, including systemic lupus erythematosus (SLE) or myasthenia gravis (MG). Aqp4-reactive T cells have also been demonstrated in NMO, but their exact pathogenic role is currently unclear. Therapies aiming at reducing circulating anti-Aqp4 antibodies such as plasma exchange, intravenous immunoglobulins and in particular the B cell-depleting anti-CD20 antibody rituximab have been shown to have a positive effect on NMO.

Infections as Triggers of MS and NMO

Despite the distinct pathophysiological mechanisms, infections have been implicated as triggers for both MS and NMO. In more general terms, autoimmune diseases are often thought to be triggered by infections. The mechanisms can be multiple, and they have all been implicated in MS and its experimental model, experimental autoimmune encephalomyelitis (EAE). *Superantigens* are infectious agent-derived substances that can activate a larger number of T (or sometimes B) cells bearing the same receptor. Superantigens have been linked to MS and EAE, in particular in the generation of relapses, which may be cytokine mediated [11, 13]. *Bystander activation* and exposure of otherwise sequestered autoantigens due to infection-induced tissue damage and subsequent epitope spreading is another possible mechanism [14]. Also, some infections act through *disruption of immune regulatory mechanisms*. Activation of innate immune receptors by pathogen-associated molecular patterns (PMP), for example, activation of TLR2 by its ligands that mimic bacterial infections, transforms regulatory T cells that are normally anti-inflammatory into cells that actually mediate inflammation by producing interleukin-17 (IL-17) [15]. The process may be mediated by infection-induced IL-6 and is more prominent in MS than in a healthy volunteer [16].

Molecular Mimicry: E. coli *and* Clostridia *in NMO as Examples*

Finally, the concept of *immunological cross-reactivity*, closely linked to that of *molecular mimicry*, explains how some infections trigger autoimmunity. Molecular similarity between components of infectious agents and self-antigens can lead to a strong cross-reactive immune response (humoral or cellular) and autoimmune disease. The clearest and best-established example is the molecular similarity between *Campylobacter* lipopolysaccharide and gangliosides present on nerve roots; antibodies against *Campylobacter* triggered by gastrointestinal infections with this agent cross-react with gangliosides and lead to the peripheral nerve-directed autoimmunity seen in some forms of acute inflammatory demyelinating polyradiculoneuropathy, or

Guillain-Barre syndrome. This topic is discussed in detail in the chapter on Guillain-Barre syndrome and *Campylobacter jejuni* enteritis.

Molecular mimicry has been explored extensively in MS. Of note is that when eluting peptides from the MS-associated major histocompatibility complex (MHC) class II MBP-presenting groove (DRB1*1501), Wuchepfenning and colleagues found that some of these peptides were derived from EBV, the infectious agent most consistently associated with MS. Some of these peptides were able to stimulate myelin basic protein-specific T cells from MS patients [17].

Molecular Mimicry in NMO

Molecular mimicry and immunological cross-reactivity have been strongly implicated in NMO.

An early study exploring this phenomenon was performed by Ren and colleagues in 2012 [18]. Since water channels (aquaporins) are expressed in most species, they searched for sequence homologies between Aqp4 and *E. coli* aquaporin-Z (AqpZ). Some regions of relevance to the immune response to Aqp4 in NMO, possibly representing B cell or T cell epitopes (the latter from experimental models), had high homology. The authors raised sera against AqpZ in mice, which showed high reactivity against both AqpZ and Aqp4 in multiple assays. Sera from patients with NMO showed strong reactivity against AqpZ protein. Moreover, anti-AqpZ antibodies were demonstrated to have cytotoxic activity against astrocytes in culture. Also, intracerebral injection of antibodies against the peptide AqpZ 174–190 induced inflammation in the CNS of injected mice, associated with behavioural and clinical abnormalities (withdrawal, reduced movement). Finally, active immunisation with AqpZ in complete Freund's adjuvant (CFA) induced CNS inflammation in mice. This inflammation was comparable in composition (mainly CD3+ T cells) and location with that induced by immunisation with Aqp4. The immunisation generated AqpZ-reactive T cells that presented a Th17 phenotype, consistent with predominant involvement of Th17 cells in NMO and the more severe forms of MS [18].

Another study analysed the T cell responses to Aqp4 in NMO [19]. The rationale was that antibodies against Aqp4 in NMO patients are of the IgG1 subclass, which requires T cell help, and there is evidence for IL-17 upregulation, implying Th17 mediation, in NMO [20]. The authors found consistent strong immunoreactivity against peptide 61–80 of Aqp4. This was inhibited by blocking antibodies against HLA-DR, suggesting MHC class II dependence of these T cell responses. They found that Aqp4 p61-80 reactive T cells of NMO patients exhibited a strong Th17 phenotype. Moreover, monocytes from NMO patients also showed pro-inflammatory polarisation. When searching for peptides with proteins with sequence homology with Aq4 p61-80, the researchers found a peptide of the *Clostridium perfringens* ABC transporter. *C. perfringens* is a ubiquitous spore-forming gram-positive bacterium found in the human gut, responsible for many cases of food poisoning [21]. The homology with Aqp4 extends to the ABC transporter of other *Clostridium*

species [19]. Interestingly, in addition to its role in inducing potential autoimmunity via cross-reactivity, *Clostridium* species and other closely related have been associated with skewing the cytokine response towards a Th17 type and thus facilitating inflammation and autoimmunity [22].

It is thus plausible that Aqp4 molecular mimicry in NMO stimulates the Th17 responses known to be prominent in NMO.

Aqp4 molecular mimicry was explored in another study [23]. In this study, structural neighbour searches were performed for primary, secondary and tertiary structure similarities to Aqp4. Similarities were confirmed with AqpZ of *E. coli*, but very high similarity was observed with the corn protein ZmTIP4-1. The study went on to demonstrate that NMO patient sera contained antibodies to ZmTIP4-1, which were cross-reactive with antibodies against Aqp4 [23]. Not only does this study strengthen the evidence for an environmental trigger in NMO but also supports the concept of molecular mimicry and immunological cross-reactivity as a mechanism of pathogenesis in NMO.

Other Roles of Clostridium *in Autoimmune Demyelination*

An intriguing study by Rumah and colleagues points to another role of *C. perfringens* in MS, mediated through its associated epsilon toxin (ETX), a toxin produced by the type B and type D *C. perfringens* but not by the commensal type A [24].

The authors isolated *C. perfringens* type B from the stool of a young woman with recent onset of clinically isolated syndrome (CIS), the first manifestation of MS. At that time she had actively gadolinium-enhancing lesions on the magnetic resonance imaging (MRI) brain scan, indicating an acute disruption of the blood-brain barrier (BBB). Since, in experimental models, ETX disrupts the BBB and produces inflammatory lesions in myelinated brain areas such as the corpus callosum [25, 26], and given its affinity for endothelial cells and myelinated areas of the brain [27], the authors postulated that ETX or perhaps a similar gut microbiota-derived toxin may contribute to some of nascent lesions in MS. This is in line with the observation of Prineas and colleagues that BBB disruption, demyelination and oligodendrocyte apoptosis may precede inflammatory T cell infiltration in nascent lesions [28].

The researchers went on and analysed stool samples from 30 people with MS and 31 healthy controls involved in a prospective study to look at their gut microbiota. They found a lower prevalence of the human commensal *C. perfringens* type A in MS patients compared to controls (23 % versus 52 %) [24]. This is interesting, because the presence of type A is generally associated with the absence of type B and D, the types that secrete ETX, probably due to competition.

The authors found that 10 % of the patients with MS displayed immunoreactivity to ETX based on a Western blot analysis, whereas only one of the control subjects had positive reactivity in the cerebrospinal fluid (CSF) and none of the controls had reactivity in the serum. There was typically a good correlation between the positivity of the reaction between the CSF and blood [24]. The seropositivity is postulated

to represent an underestimation of exposure to the bacterial toxin, given the high rate of seroreversion and of seronegativity following vaccinations of goats, suggesting that some samples may have been negative despite prior exposure to *C. perfringens*.

The study points to the possibility that *C. perfringens* contributes directly to the pathogenesis of MS through the action of ETX on the blood-brain barrier and myelin. The findings would support the epidemiological observations of Kurtzke and Hyllested (reviewed in [29]) who showed that the first MS epidemic in the Faroe Islands was associated with the arrival of the British troops during World War II. The pioneer MS epidemiologists also noted an increased incidence of gastrointestinal infections and argued for a faecal-oral transmission of the MS environmental (infectious) agent.

Murrell [30] then introduced the intriguing hypothesis of a connection with sheep, and one postulated sheep-associated pathogen was *C. perfringens* and its epsilon toxin ETX. This was further substantiated by the neurotoxic and endothelial cell-toxic effects demonstrated for ETX as discussed above.

Further studies will elucidate further the role of clostridia in MS. The increased interest in the gut microbiome in MS, EAE and other inflammatory diseases will facilitate further discoveries. Of interest is the observation from experimental studies in mice that the segmented filamentous bacteria or other similar bacteria that contribute to the pathogenicity and pro-inflammatory, predominantly Th17, profile in experimental autoimmune disease are thought to be spore forming and related to clostridia [22].

Clostridium difficile

C. difficile is a major cause of health concern due to its association with antibiotic-associated colitis, a major cause of morbidity and mortality worldwide. It is transmitted by faecal-oral route and is established in the human colon in 2–5 % of the population. It is unclear whether patients with MS and other inflammatory demyelinating diseases are colonised in a higher proportion compared to the general population, but since colonisation is associated with lengthy hospitalisation or nursing home residence, it is conceivable that MS patients, especially at more advanced stages, are more likely to be colonised. The implications of this infection are very important. This group of patients is more likely to receive antibiotics and to have bowel dysfunction (as discussed in the chapter on the impact of MS on the gastrointestinal function in this book).

Faecal transplantation is advocated and employed increasingly for severe, refractory *C. difficile* colitis, and its spectrum of application has expanded beyond the primary indication of colitis [31]. Anecdotal reports note the success of the intervention in people with MS.

Clostridium difficile infection can complicate autologous stem cell transplantation in MS, as discussed below.

The Gut Microbiome in MS and Its Relationship with MS Treatment

The gut microbiome and its role in MS have been receiving increasing attention in the recent years, since the observations that manipulations of the gut flora can modulate experimental MS-like inflammatory demyelination [32]. The gut microbiome and the effect of its modulation on clinical and experimental MS are discussed in separate dedicated chapters in this book.

Here, we only discuss the potential effects of MS therapies on the gut flora and the implications for MS.

The effect of the most commonly used MS drugs on the gut flora or MS patients is largely unknown and studies are in their infancy. Cantarel and colleagues have published pilot data comparing the gut microbiome of MS patients and healthy volunteers and also studied the effect of treatment with glatiramer acetate or vitamin D [33]. They noted considerable overlap between operational taxonomic units between MS patients and controls, but *Faecalibacterium* was less represented in MS patients. Specific changes were observed after treatment with glatiramer acetate and vitamin D [33]. Although this study was very exploratory and future studies in larger populations are needed to confirm the findings, the results indicate that the gut flora in MS is subject to modulation by drugs used in the treatment of MS.

Important recent experimental studies in cancer have pointed out the role of the gut flora in determining the response to cancer chemotherapeutic agents, notably cyclophosphamide [34]. The antitumour immune response and the efficacy of cyclophosphamide are influenced by the gut flora, and antibiotics compromise the antitumour effects of chemotherapy [34, 35]. Since cyclophosphamide is a drug that has been used in the treatment of MS [36] and also is used in bone marrow ablation prior to haematopoietic stem cell transplantation (HSCT), the evidence that changes in the gut microbiota induced, for example, by antibiotics can influence its effect needs to be considered further in MS. In addition, whether other immunosuppressive treatments used in MS are subject to the same influences remains to be determined.

In the recent months, autologous HSCT, particularly the non-myeloablative type [37], has been reported to be a promising treatment for aggressive forms of MS. Interestingly, a population of immune cells that is significantly depleted by the treatment are the IL-17-producing, pro-inflammatory mucosal-associated invariant T cells (MAIT) [38]. Targeting these cells, with potential beneficial effects in EAE, can also be achieved through manipulations of the gut flora.

It is important to note, however, that the rate of *C. difficile* infections seems to be higher in MS patients than in patients receiving the transplantation for haematological malignancies. This may reflect the longer hospitalisation in part due to more challenging mobilisation following the procedure.

Awareness of the possibility of *C. difficile* infections which may complicate HSCT is therefore crucially important [39].

Summary and Conclusions

This chapter has reviewed the evidence of the role of infections, with particular attention to the infections with gut pathogens, in NMO and MS. The evidence of molecular mimicry between the known autoantigen for NMO, Aqp4 and proteins from gut bacteria such as *E. coli* AqpZ and *Clostridium* ABC transporters, as well as some plant aquaporins, has been discussed. The functional consequence of this mimicry may be immunological cross-reactivity triggering autoimmune damage. The evidence for molecular mimicry is less robust in MS, at least in part due to the fact that the autoantigen is not as clearly established as for NMO, and the possibility that there are several autoantigens.

Direct effects of substances produced by gut bacteria such as clostridia may also be involved in the pathogenesis of MS. The epsilon toxin of *C. perfringens* is a candidate for a contribution to the pathology of some of the nascent lesions, supported by studies showing its neurotoxic effects and by epidemiology data implicating in triggering MS.

This chapter does not deal with the gut flora in MS or NMO, as this is addressed in other chapters. The manipulation of the gut microbiota can have therapeutic effects in MS. This chapter discusses briefly the opposite regulation: the potential effects of MS treatment on the gut microbiota. The role of gut flora in the effects of cyclophosphamide as shown in experimental cancer studies suggests that the issue needs to be considered when using this drug as MS treatment. A potential promising treatment, HSCT, which in part works through depletion of MAIT, may carry the risk of *C. difficile* infection, which needs to be taken into account when pondering such powerful treatment.

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References

1. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502–17.
2. Mackenzie IS, Morant SV, Bloomfield GA, MacDonald TM, O'Riordan J. Incidence and prevalence of multiple sclerosis in the UK 1990–2010: a descriptive study in the General Practice Research Database. J Neurol Neurosurg Psychiatry. 2014;85(1):76–84. Pubmed Central PMCID: 3888639.
3. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, Hellenthal G, Pirinen M, Spencer CC, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476(7359):214–9. Pubmed Central PMCID: 3182531.
4. Hartmann FJ, Khademi M, Aram J, Ammann S, Kockum I, Constantinescu C, et al. Multiple sclerosis-associated IL2RA polymorphism controls GM-CSF production in human TH cells. Nat Commun. 2014;5:5056.

5. van Luijn MM, Kreft KL, Jongsma ML, Mes SW, Wierenga-Wolf AF, van Meurs M, et al. Multiple sclerosis-associated CLEC16A controls HLA class II expression via late endosome biogenesis. Brain. 2015;138(Pt 6):1531–47.
6. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: noninfectious factors. Ann Neurol. 2007;61(6):504–13.
7. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol. 2007;61(4):288–99.
8. Wingerchuk DM, Weinshenker BG. Neuromyelitis optica. Curr Treat Options Neurol. 2008;10(1):55–66.
9. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet. 2004;364(9451):2106–12.
10. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med. 2005;202(4):473–7. Pubmed Central PMCID: 2212860.
11. Hinson SR, Pittock SJ, Lucchinetti CF, Roemer SF, Fryer JP, Kryzer TJ, et al. Pathogenic potential of IgG binding to water channel extracellular domain in neuromyelitis optica. Neurology. 2007;69(24):2221–31.
12. Constantinescu CS, Gran B. The essential role of T cells in multiple sclerosis: a reappraisal. Biomed J. 2014;37(2):34–40.
13. Constantinescu CS, Wysocka M, Hilliard B, Ventura ES, Lavi E, Trinchieri G, et al. Antibodies against IL-12 prevent superantigen-induced and spontaneous relapses of experimental autoimmune encephalomyelitis. J Immunol. 1998;161(9):5097–104.
14. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature. 1992;358(6382):155–7.
15. Nyirenda MH, Sanvito L, Darlington PJ, O'Brien K, Zhang GX, Constantinescu CS, et al. TLR2 stimulation drives human naive and effector regulatory T cells into a Th17-like phenotype with reduced suppressive function. J Immunol. 2011;187(5):2278–90.
16. Nyirenda M, Morandi E, Vinkemeier U, Constantin-Teodosiu D, Drinkwater S, King L, et al. Toll-like receptor 2 stimulation regulates the balance between Treg and Th17 function: a novel mechanism of reduced Treg function in multiple sclerosis. J Immunol. 2015;194(12):5761–74.
17. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell. 1995;80(5):695–705.
18. Ren Z, Wang Y, Duan T, Patel J, Liggett T, Loda E, et al. Cross-immunoreactivity between bacterial aquaporin-Z and human aquaporin-4: potential relevance to neuromyelitis optica. J Immunol. 2012;189(9):4602–11. Pubmed Central PMCID: 3586280.
19. Varrin-Doyer M, Spencer CM, Schulze-Topphoff U, Nelson PA, Stroud RM, Cree BA, et al. Aquaporin 4-specific T cells in neuromyelitis optica exhibit a Th17 bias and recognize Clostridium ABC transporter. Ann Neurol. 2012;72(1):53–64. Pubmed Central PMCID: 3405197.
20. Uzawa A, Mori M, Arai K, Sato Y, Hayakawa S, Masuda S, et al. Cytokine and chemokine profiles in neuromyelitis optica: significance of interleukin-6. Mult Scler. 2010;16(12):1443–52.
21. Lindstrom M, Heikinheimo A, Lahti P, Korkeala H. Novel insights into the epidemiology of Clostridium perfringens type A food poisoning. Food Microbiol. 2011;28(2):192–8.
22. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139(3):485–98. Pubmed Central PMCID: 2796826.
23. Vaishnav RA, Liu R, Chapman J, Roberts AM, Ye H, Rebolledo-Mendez JD, et al. Aquaporin 4 molecular mimicry and implications for neuromyelitis optica. J Neuroimmunol. 2013;260(1–2):92–8. Pubmed Central PMCID: 3682654.

24. Rumah KR, Linden J, Fischetti VA, Vartanian T. Isolation of Clostridium perfringens type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. PLoS One. 2013;8(10):e76359. Pubmed Central PMCID: 3797790.
25. Finnie JW. Ultrastructural changes in the brain of mice given Clostridium perfringens type D epsilon toxin. J Comp Pathol. 1984;94(3):445–52.
26. Finnie JW. Histopathological changes in the brain of mice given Clostridium perfringens type D epsilon toxin. J Comp Pathol. 1984;94(3):363–70.
27. Zhu C, Ghabriel MN, Blumbergs PC, Reilly PL, Manavis J, Youssef J, et al. Clostridium perfringens prototoxin-induced alteration of endothelial barrier antigen (EBA) immunoreactivity at the blood–brain barrier (BBB). Exp Neurol. 2001;169(1):72–82.
28. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol. 2004;55(4):458–68.
29. Kurtzke JF. Epidemiology in multiple sclerosis: a pilgrim's progress. Brain J Neurol. 2013;136(Pt 9):2904–17.
30. Murrell TG, O'Donoghue PJ, Ellis T. A review of the sheep-multiple sclerosis connection. Med Hypotheses. 1986;19(1):27–39.
31. Xu MQ, Cao HL, Wang WQ, Wang S, Cao XC, Yan F, et al. Fecal microbiota transplantation broadening its application beyond intestinal disorders. World J Gastroenterol WJG. 2015;21(1):102–11. Pubmed Central PMCID: 4284325.
32. Yokote H, Miyake S, Croxford JL, Oki S, Mizusawa H, Yamamura T. NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. Am J Pathol. 2008;173(6):1714–23. Pubmed Central PMCID: 2626383.
33. Cantarel BL, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, et al. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. J Invest Med 63(5):729–34.
34. Viaud S, Daillere R, Boneca IG, Lepage P, Langella P, Chamaillard M, et al. Gut microbiome and anticancer immune response: really hot Sh*t! Cell Death Differ. 2015;22(2):199–214. Pubmed Central PMCID: 4291500.
35. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science. 2013;342(6161):967–70.
36. Awad A, Stuve O. Cyclophosphamide in multiple sclerosis: scientific rationale, history and novel treatment paradigms. Ther Adv Neurol Disord. 2009;2(6):50–61. Pubmed Central PMCID: 3002608.
37. Burt RK, Balabanov R, Han X, Sharrack B, Morgan A, Quigley K, et al. Association of nonmyeloablative hematopoietic stem cell transplantation with neurological disability in patients with relapsing-remitting multiple sclerosis. Jama. 2015;313(3):275–84.
38. Abrahamsson SV, Angelini DF, Dubinsky AN, Morel E, Oh U, Jones JL, et al. Non-myeloablative autologous haematopoietic stem cell transplantation expands regulatory cells and depletes IL-17 producing mucosal-associated invariant T cells in multiple sclerosis. Brain J Neurol. 2013;136(Pt 9):2888–903. Pubmed Central PMCID: 3754461.
39. Burt RK, Loh Y, Cohen B, Stefoski D, Balabanov R, Katsamakis G, et al. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. Lancet Neurol. 2009;8(3):244–53.

Chapter 14
Targeting Immunomodulatory Agents to the Gut-Associated Lymphoid Tissue

Atheer Zgair, Jonathan Chi Man Wong, and Pavel Gershkovich

Abstract In addition to fluid haemostasis and lipid absorption, the lymphatic system and lymphoid tissues serve as the major host of immune cells where immune responses are evoked. Impaired function of the immune system might lead to serious diseases which are often treated by immunomodulators. This chapter briefly explores the physiology of an important part of the lymphatic system, the gut-associated lymphoid tissues (GALT). Currently used strategies for targeting GALT by immunomodulators for enhanced activity and/or decreased side effects are discussed. Strategies range from simple oral co-administration of immunomodulators with lipids to more advanced lipid-based formulations, polymer-based nanoparticle formulations and prodrugs. These targeting approaches successfully increase the concentration of immunomodulators achieved in the GALT and, more importantly, enhance immunomodulatory effects. Therefore, targeting immunomodulators to GALT represent a promising approach in the treatment of diseases where the immune system is actively involved.

Keywords GALT • Immunomodulators • Targeting strategies • Lipid-based drug delivery systems • Nanoparticles • Prodrugs • Long-chain triglycerides Chylomicrons • Cannabinoids • Autoimmune disorders

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Introduction

The current understanding of the lymphatic system and lymphoid tissues was developed over the centuries. In the fourth century B.C., components of the lymph system, particularly the auxiliary lymph nodes, were first described by Hippocrates as 'vessels containing white blood' [1]. However, it was not until the sixteenth century A.D. when the Italian physician Aselli succeeded to describe the lymphatic system in the gut of fed dogs. This discovery was made accidentally while trying to observe the diaphragm. He noticed a network of vessels containing milky white fluid that he later named 'lacteal vessels'. However, Aselli suggested that these vessels deliver their contents to the liver. It took many decades until the French physician Pecquet identified the thoracic ducts and proved that these ducts receive flow from the lacteals discovered by Aselli [1–4]. More accurate anatomical descriptions were developed later, using wax injections to uncover parts of the system. This included the discovery of lymph nodules in the mucous membrane of the small intestine, Peyer's patches (PP), which were named after their discoverer Johann Conrad Peyer [1, 3].

Accurate functional description of the discovered structures was not proposed until the publication of William Hunter's research. It was suggested in this research that lymphatics and lacteals are structural units of one large system distributed in all remote parts of the body [1–3]. Indeed, the lymphatic system was thought to be merely a drainage system for fluids and proteins from interstitial space back to the blood [5]. Currently, however, the lymphatic system is considered to have a central role in the pathogenesis of several diseases such as cancers, viral infections, some parasitic infections and autoimmune disorders. In fact, it is the main pathway for the metastases of some epithelial origin solid tumours, such as those of the colon, breasts, lungs and prostate [6]. In addition, the lymphatic system is now recognised as a crucial part of the immune system. It is here where invader antigens are trapped, processed and presented to immune cells and consequently where immune responses are evoked [1]. These responses are important for the protection of the body from bacterial, viral, parasitic and fungal threats, as well as the growth of tumour cells [7]. Deficiencies in the immune responses, whether inherited or acquired, weaken the body's defence mechanisms. On the other hand, over-reactive immune responses might cause life-threatening diseases, commonly called autoimmune diseases. Therefore, substances that can positively or negatively modify weak or over-reactive immune responses, respectively, provide a novel approach in the treatment of disorders where the immune system has a central role. These substances are collectively referred to as immunomodulators [8].

The last decade had witnessed the use of immunomodulators as promising therapeutic agents in the treatment of infectious diseases, autoimmune diseases and cancers and for prevention of organ transplant rejection. The therapeutic effects of immunomodulators can be achieved by either augmenting or suppressing the activity of immune cells [9, 10]. Since the lymphatic system is the major host of immune cells, the focus of this chapter is to highlight the current strategies of targeting immunomodulators, in particular cannabinoids, to the gut-associated lymphoid tissue (GALT).

Functions of the Lymphatic System

As mentioned above, a substantial interest was developed in the nineteenth century to elucidate the functions of the lymphatic system. These functions can be summarised as follows:

Fluid Recovery

Fluids continuously escape from blood capillaries to the surrounding tissues. However, a significant proportion of these fluids cannot be reabsorbed by venous capillaries. Indeed, up to four litres of fluids and half of all plasma proteins can extravasate each day. This in turn could lead to circulatory failure and increased tissue pressure if unrecovered. The lymphatic system, therefore, maintains the body's fluid balance by reabsorption of the extravasated fluids and proteins back to the systemic circulation [2, 11, 12].

Lipid Absorption

The intestinal lymphatic system has an essential physiological role in the absorption of dietary lipids and lipid-soluble vitamins [2, 11]. The first step in the absorption of dietary lipids is their digestion and micellar solubilisation in the gastrointestinal lumen. This happens mainly by the action of pancreatic lipase/co-lipase complex and bile salts in the small intestine. Once digested, the products of lipid hydrolysis are then incorporated into mixed micelles, which promote the diffusion of digested lipids to the apical membrane of enterocytes [13]. Inside enterocytes, most of the long-chain triglycerides (LCT) are resynthesised from long-chain fatty acids and monoglycerides, mainly by the action of acyltransferases. LCT are then assembled with apolipoprotein B (Apo B), phospholipids, cholesterol and cholesterol esters to form large lipoproteins with a lipid core (chylomicrons, CM). Mature CM are then secreted by exocytosis through the basolateral membrane of enterocytes. Being large particles, CM cannot pass the walls of vascular capillaries but are absorbed to the lymph lacteals instead [14–17]. Because of the presence of lipids in the form of CM, lymph fluid following high-fat meal looks like a turbid emulsion which is commonly called 'chyle' [6].

Immunity

The immune system is not a definite organ system per se, but rather a population of cells distributed in all organs to defend the body against any potential invaders. The most important cells involved in immune responses are lymphocytes. Over 90 % of lymphocytes are localised in the lymphatic system [11, 18].

When collecting fluid and plasma proteins, the lymphatic system also picks up foreign bodies from tissues. These bodies are drained along the lymph to the regional lymph nodes where immune cells can initiate an immune response. Therefore, lymph nodes stand as checkpoints that examine lymph fluid before it is drained to the bloodstream [11].

Components of the Lymphatic System

Lymph

Lymph is usually a clear and colourless fluid which is drained from the interstitium. In addition to the recovered fluids and plasma proteins, lymph may also contain lipids, immune cells, hormones, bacteria, viruses, cellular debris or even cancer cells. Substantial differences in lymph composition arise from physiological and/or pathological conditions of the tissue from which lymph is drained, as well as its location along the lymphatic vessels [11, 19].

Lymphatic Vessels

The lymphatic system is the body's second circulatory system. However, unlike the closed structure of the blood vessels, the lymphatic system consists of unidirectional, blind-ended and thin-walled capillary vessels where lymph is driven without a central pump [5, 20, 21]. Lymphatic capillaries drain in the afferent collecting vessels, which then pass through one or more gatherings of lymph nodes. Lymph fluid then passes through the efferent collecting vessels, larger trunks and finally the lymphatic ducts. Subsequently, ducts drain lymph to the systemic circulation [6, 22].

Lymphatic Organs

The lymphatic organs can be classified as primary or secondary. Primary lymphatic organs include the thymus gland and bone marrow, which produce mature lymphocytes (that can identify and respond to antigens). Secondary lymphatic organs include lymph nodes, spleen and mucosa-associated lymph tissues (MALT) [23–25]. It is within the secondary lymphatic organs that lymphocytes initiate immune responses. MALT are distributed throughout mucous membranes and provide a defence mechanism against a wide variety of inhaled or ingested antigens. MALT can be categorised according to their anatomical location to bronchus-associated lymphoid tissue (BALT), nasal-associated lymphoid tissue (NALT), salivary gland duct-associated lymphoid tissue (DALT), conjunctiva-associated lymphoid tissue

(CALT), lacrimal duct-associated lymphoid tissue (LDALT) and gut-associated lymphoid tissue (GALT) [23, 26].

Gut-Associated Lymphoid Tissue (GALT)

GALT consists of effector and immune induction sites. The former is represented by lymphocytes distributed throughout the lamina propria (LP) and intestinal epithelium, while the latter involves organised tissues such as mesenteric lymph nodes (MLN), PP and smaller isolated lymphoid follicles (ILF) [27–30]. Some authors, however, define MLN as separate lymphatic organs rather than a part of GALT [31, 32]. In this chapter, MLN are included when referring to the GALT.

Mesenteric lymph nodes (MLN) are the largest gatherings of lymph nodes in the body, found in the base of the mesentery. The structure of MLN is similar to that of peripheral lymph nodes and can be divided into two regions: the medulla and cortex. The cortex is mainly composed of T-cell areas and B-cell follicles. It is within the T-cell area where circulating lymphocytes enter the lymph node and dendritic cells (DC) present antigens to T-cells [17, 33, 34]. Lymph (containing cells, antigens and chylomicrons) is collected from the intestinal mucosa and reaches MLN via the afferent lymphatics. Lymph fluid subsequently leaves MLN through efferent lymphatics to reach the thoracic duct that drains to the blood [27, 34].

Peyer's patches (PP) are a collection of lymphoid nodules distributed in the mucosa and submucosa of the intestine. They consist of a sub-epithelial dome area and B-cell follicles dispersed in a T-cell area. A single layer of epithelial cells, called follicle-associated epithelium (FAE), separates lymphoid areas of PP from the intestinal lumen. FAE is permeated by specialised enterocytes called microfold (M) cells. These cells are considered as a gate for the transport of luminal antigens to PP [27, 30].

Isolated lymphoid follicles (ILF) are a combination of lymphoid cells in the intestinal LP. ILF are structurally similar to PP in the sense that they are composed of germinal centre covered by FAE containing M-cells. However, unlike PP, ILF lack a discrete T-cell area. Although its function is not completely understood, ILF is thought to be a complementary system to PP for the induction of intestinal immunity [32, 35].

It is noteworthy that GALT is the largest lymphatic organ in the human body and contains more than half of the body's lymphocytes [36, 37]. GALT is also exposed to more antigens than any other part of the body, in the form of commensal bacteria and alimentary antigens, in addition to those from invasive pathogens. The intestinal immune system must therefore be able to distinguish antigens that require a protective immune response and to develop a state of immune hypo-responsiveness (oral tolerance) for those antigens that are harmless to the body [27, 30, 32]. The mechanism governing this process involves sampling of luminal antigens in the intestinal epithelium by DC. Antigens can cross the epithelium through M-cells that are found in the FAE of PP. The antigens can then interact with DC in the underlying sub-epithelial dome region. Antigens are then presented to local T-cells in PP by DC. DC

can also migrate to the draining MLN where they present antigens to local lymphocytes [23, 27, 30, 38]. Alternative pathways for antigen transport across the intestinal epithelial cells involve receptor-mediated transport, as well as direct sampling from the lumen by DC's projections. Antigen-loaded DC then migrate to the MLN through afferent lymphatics where they present antigens to T-cells. Subsequently, differentiated lymphocytes migrate from MLN through the thoracic duct and blood stream and eventually accumulate in the mucosa for an appropriate immune response (Fig. 14.1) [27, 39].

Targeting GALT

In general, GALT could be a target (effective compartment) and/or a route through which therapeutic agents are delivered to the systemic circulation.

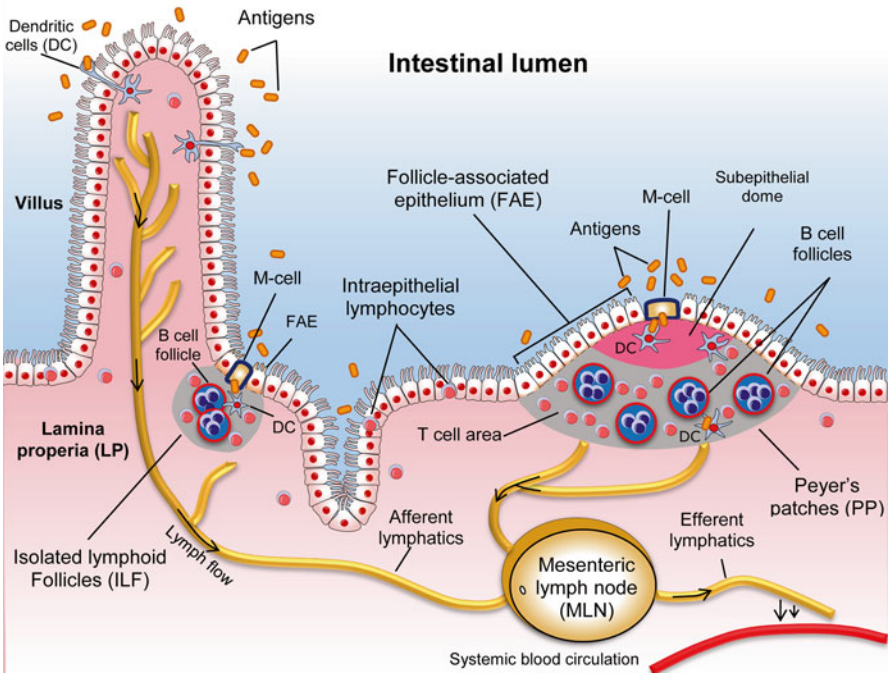

Fig. 14.1 Schematic representation of the gut-associated lymphoid tissue (GALT). Dendritic cells (*DC*) can sample luminal antigens that (1) cross M-cells of Peyer's patches (*PP*) and isolated lymphoid follicles (*ILF*) and (2) transported to lamina propria (*LP*) by receptor-mediated mechanisms. In addition, DC can use trans-epithelial projections to sample antigens directly from the lumen. DC then present antigens to local lymphocytes or migrate to mesenteric lymph nodes (*MLN*) for lymphocyte priming

Advantages of Targeting GALT

- Achieving high local concentration in the GALT could be of particular importance for pharmacological agents such as immunomodulators, for example, cannabinoids, some chemotherapeutic agents and anti-infective agents, thereby decreasing dose-related systemic side effects as well as systemic dilution [6, 18]. The lymphatic system is a main pathway of intestinal tumour metastases; therefore, targeting cytotoxic drugs to the intestinal lymphatics could provide advantage in the treatment of tumour metastases [40, 41]. Being the largest lymphatic organ, GALT provide a valid delivery target for antiviral agents, as some viruses spread and develop within the lymphatic system. Those of particular importance are human immunodeficiency virus (HIV) morbillivirus, canine distemper virus, severe acute respiratory syndrome (SARS)-associated coronavirus, hepatitis B and hepatitis C [42].
- Increasing the bioavailability of lipophilic drugs when orally co-administered with lipid vehicles could be another advantage. This primarily occurs as a result of enhancing micellar solubilisation of the drug in the small intestine and drug-CM association in enterocytes [43]. One important reason is that intestinal lymphatic transport avoids hepatic first-pass metabolic loss by diverting the absorption of lipophilic drugs towards intestinal lymphatics rather than the portal vein, which is extremely important for drugs exhibiting significant first-pass metabolism [16].
- Intestinal lymphatic transport of lipophilic drugs results in delivery of the drug to the systemic circulation in CM-associated form, which might attenuate the pharmacokinetic and/or pharmacodynamic properties [41, 44].

Miura et al. [34] have shown that oral administration of the LCT (olive oil) can enhance lymphocyte transport in mesenteric lymphatics of rats more than tenfold. Miura et al. also demonstrated that the enhancement of lymphocyte flux was selective to the administration of the long-chain but not the medium-chain fatty acids. This in turn was secondary to the assembly of CM by enterocytes to enhance the absorption of orally administered long-chain fatty acids, as the co-administration of Pluronic L-81 (an inhibitor of intracellular CM transport and secretion) significantly decreased lymphocyte transport (Fig. 14.2). Moreover, the effect of long-chain fatty acids (particularly the monounsaturated fatty acids) was not limited to the augmentation of lymphocyte flux, but also stimulated lymphocyte proliferation. The precise mechanism governing these effects is unclear. However, a mechanism that involves the utilisation of CM's phospholipids and fatty acids has been suggested [37, 45]. This view is supported by the observations by Calder et al. [46] who found that lymphocytes have lipoprotein lipase activities and are able to release fatty acids from triglycerides (TG) present in CM and very low-density lipoproteins. In addition, the study also showed that TG rich in polyunsaturated fatty acids (PUFA) such as linoleic acid are potent inhibitors of lymphocyte proliferation in vitro, while this effect was not observed when using the monounsaturated oleic acid. This can in part explain the clinical benefits of daily administration of vegetable oils containing linoleic acid to patients suffering from inflammatory and autoimmune diseases,

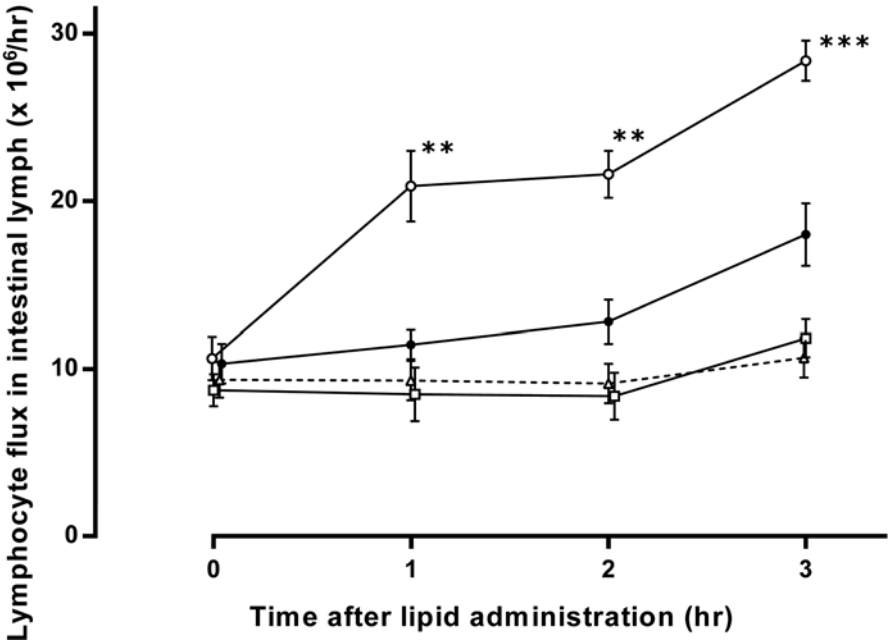

Fig. 14.2 Lymphocyte flux of intestinal lymph (mean ± SEM, $n = 6$) after administration of oleic acid (○—○), octanoic acid (□—□), oleic acid with Pluronic L-81 (●—●) and control (sodium taurocholate, Δ----Δ) into the duodenum of lymph-fistulated rats. One-way ANOVA was used to assess statistical differences from the control values. ** $p < 0.01$; *** $p < 0.001$ (Reproduced with permission from Miura et al. [45])

such as rheumatoid arthritis, psoriasis and multiple sclerosis [46, 47]. Therefore, it is reasonable to conclude that targeting of immunomodulators to GALT could present a valid treatment strategy for a wide range of serious diseases.

Strategies for Targeting the GALT

Lipid-Based Drug Delivery Systems (LBDDS)

In a list of the top 200 marketed orally administered drugs, up to 40 % are poorly water-soluble, which is usually associated with poor absorption by the gut and low bioavailability [48]. Many biologically active drugs are also highly metabolised before they are able to exert their beneficial effect, which also reduces bioavailability. The combination of poor absorption and high first-pass metabolism has created the need for drug delivery systems that can improve the absorption of poorly water-soluble drugs and also protect them from degradation. One of the most promising strategies to address these issues is LBDDS [49–51].

Co-administration with Lipids

The simplest method of targeting drugs to the GALT is by co-administration of lipids with the drug. Oral administration of lipids can change the pharmacokinetic and pharmacodynamic profiles of drugs [52] by reducing the rate of gastric emptying and stimulating the release of bile (containing surfactants such as bile acids and phospholipids) from the gall bladder. Bile acids are hydrophilic on one end and hydrophobic on the other, whereas phospholipids generally have one hydrophilic tail and two hydrophobic tails (two fatty acids). These amphiphilic surfactants emulsify the lipids to form a fine emulsion, which prevents the droplets from aggregating back into larger particles. This emulsification vastly increases the surface area of the lipids and provides an interface for pancreatic lipase/co-lipase complex to digest them [53].

The digestion of TG by pancreatic lipase forms monoglycerides and fatty acids, as previously described in this chapter. TG, cholesterol and cholesterol esters are transported by CM, which typically vary from 75 to 1200 nm in diameter [54]. After leaving the enterocyte, CM are unable to enter the portal circulation due to their large size and enter the lymphatics instead, which allows passage of large particles [55]. Some drugs can exploit this transport pathway by associating with fatty acids and TG at any of the aforementioned steps, ending up inside the core of CM. Due to the hydrophobic nature of this core, highly lipophilic drugs are very good candidates to be transported via this pathway. Drug candidates for intestinal lymphatic transport have been classically described as having a water-to-octan-1-ol partition coefficient (log *P*) higher than 4.7 and TG solubility higher than 50 mg/mL [16, 56]. More recently other physicochemical properties have been included, most notably a drug's distribution coefficient at pH 7.4 (log $D_{7.4}$) [57].

An example of immunomodulatory drug targeted to the GALT by co-administration with lipids is JWH-015. This drug is an investigational lipophilic cannabinoid 2 (CB_2) receptor agonist that has immunomodulatory effects [58] and therapeutic benefits in animal model of multiple sclerosis [59]. Cannabinoids in general are a group of chemical compounds that act on cannabinoid receptors and have been reported to have immunomodulatory effects [60]. In a study by Trevaskis et al. [18], the intestinal lymphatic transport and the recovery of JWH-015 in the collected lymph lymphocytes were assessed in mesenteric lymph duct-cannulated rats following intraduodenal infusion with oleic acid. In this study, JWH-015 was administered in lipid formulations containing either 4 or 40 mg oleic acid. The authors concluded that proportions of JWH-015 doses recovered in the mesenteric lymph and lymphocytes were significantly higher (53 and 176 fold, respectively) following the administration of 40 compared to 4 mg oleic acid formulations. Although lymphocyte flux into the mesenteric lymph was elicited by as low as 4 mg oleic acid, high lipid formulation (40 mg oleic acid) increased lymphocyte flux up to fivefold. Thus, in this study, co-administration of JWH-015 with long-chain fatty acids affected GALT's lymphocytes by three mechanisms: enhancement of drug absorption from the intestinal lumen, stimulation of the intestinal lymphatic transport of the drug and increase in lymphocyte flux to the area. Furthermore, Trevaskis et al. compared the lymphatic

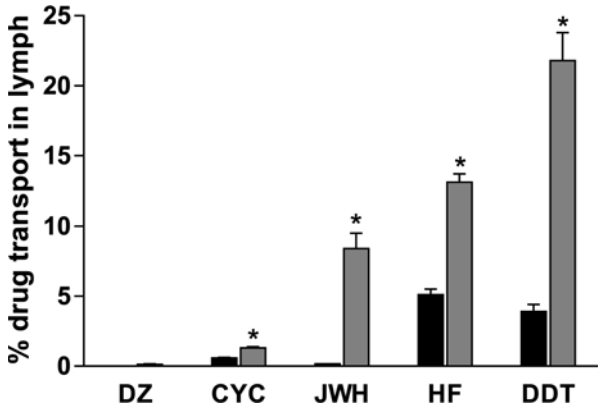

Fig. 14.3 Effect of drug lipophilicity and co-administration of 4 mg (filled bars) and 40 mg oleic acid (open bars) on the extent of intestinal lymphatic transport (mean ± SEM, $n = 4$ or 5) in mesenteric lymph duct-cannulated rats. One-way ANOVA with Tukey's post hoc test was used for statistical analysis. * Significantly higher than 4 mg of lipid group. *DZ* diazepam, *CYC* ciclosporin, *JWH* JWH-015, *HF* halofantrine, *DDT* dichlorodiphenyltrichloroethane (Reproduced with permission from Trevaskis et al. [18])

transport of JWH-015 to that of other model lipophilic molecules, namely, dichlorodiphenyltrichloroethane (DDT), halofantrine, ciclosporin and diazepam which are insecticidal, antimalarial, immunosuppressant and central nervous system depressant drugs, respectively. The magnitude of the intestinal lymphatic transport correlated with the lipophilicity and TG solubility of these drugs. The results showed that the extent of intestinal lymphatic transport was enhanced when the drug was co-administered with 40 compared to 4 mg oleic acid for all drugs (Fig. 14.3).

Dexanabinol is another non-psychotropic synthetic cannabinoid that has been suggested to have therapeutic immunomodulatory effects in the treatment of experimental multiple sclerosis [61]. Gershkovich et al. [62] evaluated the lymphatic transport of dexanabinol following oral administration in LCT-based formulation in rats. The authors found that the concentration of dexanabinol recovered in the mesenteric lymph was around 80-fold higher than that in plasma. In the same study, another, more lipophilic cannabinoid (PRS-211,220) has been found to have more than 550-fold higher concentrations in the mesenteric lymph versus plasma. These findings suggest that the administration of lipophilic cannabinoids with LCT is a promising targeting strategy to GALT.

Emulsions

Emulsions are defined as mixtures of two or more immiscible liquids (Fig. 14.4a). For pharmaceutical applications, emulsions are generally made from three components: oil, surfactant and water. The hydrophile-lipophile balance of these components determines whether the resulting emulsion is oil droplets in water (oil-in-water),

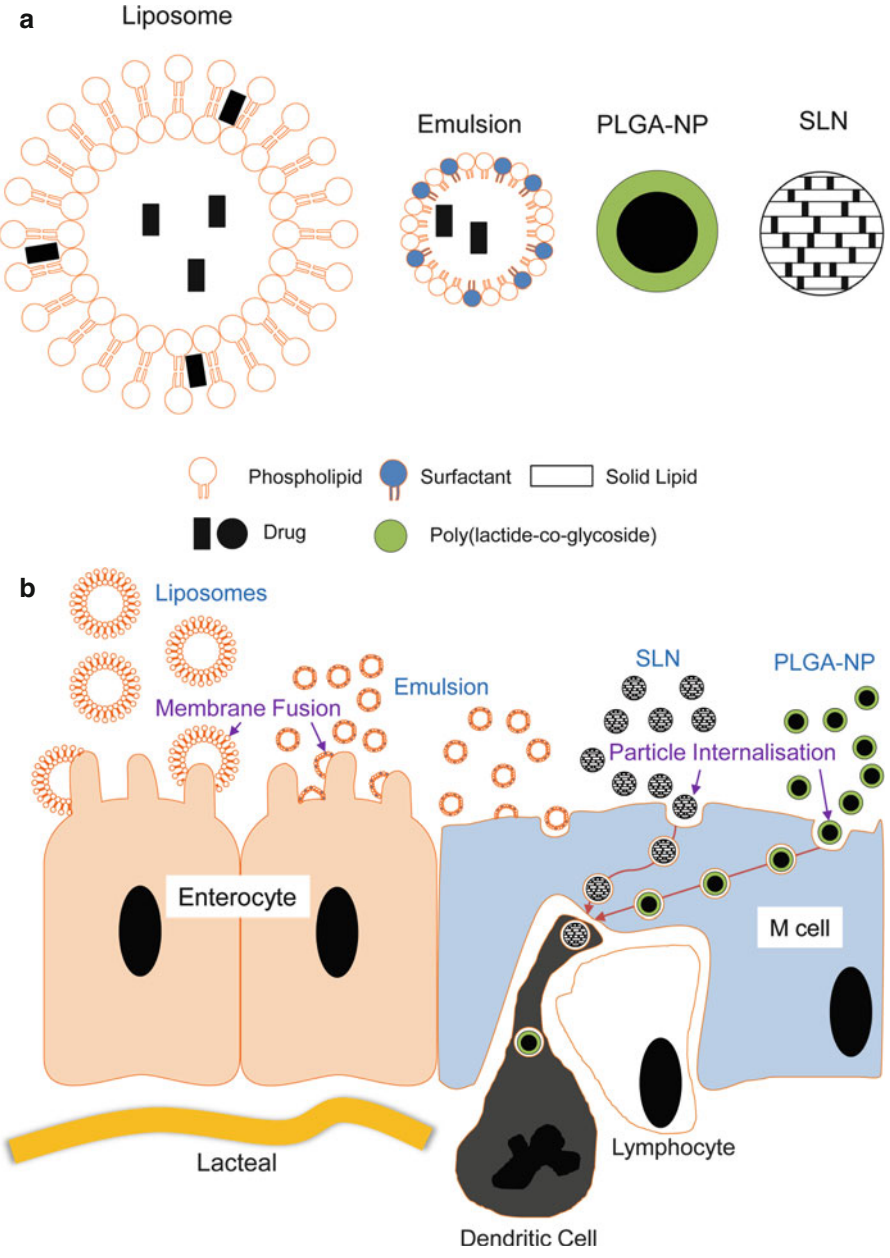

Fig. 14.4 Drug delivery systems targeting the gut-associated lymphoid tissue (GALT) and their absorption in the intestine. (**a**) The structure of unilamellar liposomes, emulsions, polymeric nanoparticles (PLGA-NP) and solid lipid nanoparticles (SLN). (b) The two main pathways of the uptake of these drug delivery systems in the intestine

water droplets in oil (water-in-oil), micelles, oily dispersions or isotropic solutions that are emulsified upon contact with water. The last of these mixtures have been termed self-emulsifying drug delivery systems (SEDDS). By forming their own emulsion, drugs delivered this way are protected from degradative enzymes [63] and are not as reliant on endogenous surfactants to increase their surface area for absorption [64, 65], while the presence of lipid within the emulsion also stimulates lymphatic transport [66].

A well-known example where an immunomodulatory agent was orally delivered in a microemulsion is ciclosporin, a polypeptide drug widely used to prevent rejection of organs after transplantation by suppressing the activity of T-cells [67]. However, ciclosporin has very low solubility in water (23 μg/mL at 20 °C) and is also extensively metabolised by cytochrome P-450 enzymes [68–71]. Substantial research has been done about formulating ciclosporin into emulsions containing lipid microspheres [67] or milk fat globule membranes [65]. Since its approval for use, a number of different formulations of ciclosporin became commercially available, many of which are emulsions, the most common being Sandimmune® and its newer formulation Neoral® [65]. Ciclosporin is an important candidate for intestinal lymphatic transport, since it is highly metabolised in the liver into metabolites with lower immunosuppressive activity [69, 70]. Sandimmune®, the original formulation of ciclosporin, vastly improved its bioavailability but had high inter- and intra-patient variability. Therefore, an optimised formulation consisting of dl-α-tocopherol, corn oil derivatives and polyoxyl 40 hydrogenated castor oil, named Neoral®, was tested and resulted in more predictable pharmacokinetic profiles and more extensive drug absorption [65, 72, 73].

Another example of an immunomodulatory drug that was targeted to the GALT using emulsion-based formulation is in work done by Zhang et al. [74]. Morin, a xanthine oxidase inhibitor which has been shown to play a role in the treatment of gout was formulated into a self-nanoemulsifying drug delivery system (SNEDDS) to improve its oral absorption. SNEDDS are made from very similar components to other emulsions but are distinct in that they are not thermodynamically stable (but kinetically stable), which means that emulsification of SNEDDS is not as affected by temperature and dilution, but the emulsion will separate into different phases after prolonged storage [74–76]. In their work, Zhang et al. conjugated a phospholipid complex to morin in addition to incorporation into SNEDDS to increase its intestinal permeability and examined the intestinal absorption and lymphatic transport of their SNEDDS compared to conjugated drug and free drug. The group showed that SNEDDS were found in the GALT after oral administration, particularly from the segments closer to the ileum, due to the presence of PP and M-cells [74].

Liposomes

Liposomes are closed spherical structures consisting of at least one phospholipid bilayer, ranging from 100 to 5000 nm. Much like other phospholipid bilayers, they are capable of containing an aqueous phase within (see Fig. 14.4a). As mentioned

earlier, the amphiphilic nature of phospholipids allows them to hold both hydrophilic (contained within the aqueous phase of the liposome) and hydrophobic (incorporated in the bilayer membrane) drugs [77]. Drugs that are incorporated in liposomes are also protected from degradation, which increases their therapeutic efficacy and reduces side effects [78].

Liposomes release their contents upon degradation in the lysosome or fusion with another lipid bilayer, such as those in the cell membrane, or phagocytes [79–81]. Liposome membranes are extremely modifiable and have been shown to deliver their encapsulated drugs by a wide number of stimuli, such as pH [82, 83], temperature [84], redox potential [85, 86], magnetism [84, 87], ultrasound [88, 89] and light [90]. Although liposomes have also been shown to successfully target different parts of the lymphatic system following various routes of administration, such as subcutaneous, pulmonary and intramuscular injection [77, 79, 81, 84, 86, 91], this section will focus on liposomes that target the GALT following oral administration. Liposomes are too large to enter the intestinal blood capillaries, which have a pore size between 60 and 80 nm [55, 92, 93], and therefore enter the lymphatics instead.

Liposomes have been used in the delivery of proteins [91–93] and DNA [94, 95]. Perrie et al. [94] incorporated plasmid DNA encoding a small region of hepatitis B surface antigen into liposomes and studied the immunisation conferred by oral administration in mice. Compared to naked DNA, mice receiving DNA delivered via liposomes showed a higher IgA response. The group went on to study gene expression in mice after oral administration of liposomes containing plasmid DNA encoding green fluorescent protein and found that liposomal delivery of plasmid DNA yielded much higher gene expression in the draining mesenteric lymph nodes than mice given naked plasmid DNA. The authors concluded that liposomes could be useful agents in lymphatic delivery of DNA [94].

Masuda et al. [95] incorporated ovalbumin as a model antigen into liposomes for oral delivery and examined their ability to induce oral tolerance in mice. Ovalbumin in liposomes of different compositions successfully suppressed proliferative responses of popliteal lymph node cells in mice, suggesting that liposomes were taken up by the lymphatics and induced tolerance to ovalbumin more effectively than ovalbumin administered in aqueous suspension [93]. Other research has focused on delivery of antigens encapsulated in liposomes to the lymphatics, but not via oral route [96].

Nanoparticles

Nanoparticles are particles smaller than 1000 nm in size and have been used in the delivery of drugs to the GALT, via uptake by ILF and PP, as mentioned previously in this chapter [97]. Although the exact mechanism of uptake is unclear, there have been many examples of nanoparticulate drug delivery systems that use this uptake pathway to access the GALT [98–102], some examples of which will be discussed in this section.

Lipid Nanoparticles

After the discovery and use of liposomes in the 1970s [103], a number of drawbacks were also discovered, such as drug leakage upon storage, physical instability, aggregation, presence of organic solvent residue, cytotoxicity and lack of cost-effective methods of high-quality production [104, 105]. Drug delivery systems based on naturally occurring lipids were then developed in order to overcome these problems. Lipid nanoparticles with a solid matrix had high drug loading, more controlled drug release profiles and better long-term stability and were more easily produced than emulsions or liposomes on a large scale [75, 104, 106]. One type of lipid-based nanoparticle that has been used to target the GALT is solid lipid nanoparticles.

Solid Lipid Nanoparticles (SLN)

SLN are usually made from biocompatible lipids and surfactants, such as tripalmitin [107, 108], tristearin, poloxamer 188 [109], dioleoylphosphatidylethanolamine, tricaprin, Tween 80 [110], glyceryl monostearate [111] and soya lecithin [112]. These components are in solid form at room temperature and can therefore be more stable and provide controlled release and more specific drug targeting compared to liposomes [113]. SLN are made of a solid lipid core and stabilised by surfactants. The loaded drug would then fit in the gaps between fatty acid chains of the lipid core (Fig. 14.4a). The size of particles in this type of formulation (20–1000 nm in diameter) allows efficient drug uptake into the intestinal lymphatic system due to the presence of lipids and their similar size to CM [114]. SLN can also be taken up by M-cells within PP, represented in Fig. 14.4b [115].

One example of the use of SLN to target the GALT is the work by Paliwal et al. [111]. The group loaded methotrexate into SLN made from glycerol monostearate, tristearin or Compritol 888 ATO, and the formulation was administered intraduodenally to rats. Methotrexate is used in the treatment of cancer and autoimmune diseases by antagonising folic acid metabolism [116, 117]. Drug concentration profiles in plasma and lymph were determined following intraduodenal administration of aqueous methotrexate solution and the four types of SLN loaded with methotrexate. The authors found that all SLN produced lead to increased drug bioavailability, a prolonged release compared to aqueous solution, and up to threefold higher plasma C_{max} values. In addition, lymphatic uptake of methotrexate was up to tenfold higher with SLN compared to aqueous solution [111].

Polymeric Nanoparticles

When nanoparticles were first developed for use in drug delivery, synthetic polymers were the preferred choice for their outer coating. This was due to the varying purity of natural polymers (such as polysaccharides and proteins) at the time and

their potential interaction and denaturation of contained drugs [118]. Among the most common and FDA-approved polymers used in polymeric nanoparticles are polyglycolic acid (PGA) and polylactic acid (PLA). PGA and PLA are considered biocompatible since they are degraded to glycolic and lactic acid, both of which are by-products of other metabolic pathways in the body [75, 119]. These polymers can also be combined into a copolymer, poly(lactide-co-glycoside) (PLGA). The ratio of PGA to PLA in PLGA can be fine-tuned to control degradation and drug release rates [118, 120, 121]. Polymeric nanoparticles could also be taken up by PP, in a manner similar to SLN [115].

Kim et al. [122] used PLGA nanoparticles entrapping type II collagen (CII) to study its ability to suppress collagen-induced arthritis in mice. Localisation of nanoparticles and CII, circulating immunoglobulin G (IgG) targeting CII, CII-specific T-cell proliferation and tumour necrosis factor α (TNFα) expression in PP and draining lymph nodes were assessed after a single oral administration of nanoparticles containing CII. The group found that CII-containing nanoparticles of 300 nm in diameter persisted in PP for 14 days after their administration and were able to reduce the incidence of arthritis by half (from 88.9 % to 43.8 %) and reduce IgG antibodies by more than half (28.6 ± 12.5 versus 78.5 ± 28.3 arbitrary units). The expression of TNFα was also up-regulated in PP cells when treated with nanoparticles, but down-regulated in the draining lymph nodes. The authors concluded that CII-containing PLGA nanoparticles were able to suppress the development of arthritis, as well as autoimmune responses.

Rebouças et al. [123] used polyanhydride nanoparticles (another biocompatible polymer) loaded with peanut extract to study oral immunotherapy for peanut allergies. Raw or roasted peanut proteins were orally administered to mice, and levels of different immunoglobulins (IgG, IgE, IgA), secretion of specific cytokines related to the immune response and the stimulation of T-helper cells were assessed. Polyanhydride nanoparticles containing peanut proteins were able to increase the production of IgG, IgE and IgA (compared to free protein and unloaded nanoparticles) and also up-regulate the expression of interleukin 10, an immunosuppressive cytokine that can reduce inflammation at sites of allergic reactions [124]. Finally, the nanoparticles were also shown to stimulate the appropriate prophylactic T-helper cell response twofold higher than free protein. While the effects of these nanoparticles in sensitised animals were not examined, the authors concluded that polyanhydride nanoparticles could be useful in food oral immunotherapy.

Prodrugs

A prodrug is a bio-reversible precursor of a drug that has an obstacle attenuating its therapeutic efficacy [125]. In regard to GALT targeting, prodrug can modify a drug's physicochemical properties in such a way that would improve its delivery to the GALT. This can be achieved by a mechanism that involves the association of the prodrug with CM assembled in enterocytes. Therefore, a prodrug can be designed to have certain physicochemical properties, particularly log $D_{7.4} \geq 5$ and

high lipid solubility [16, 126], so that its intestinal lymphatic transport is enhanced when the prodrug is co-administered orally with LCT. Alternatively, a prodrug can be structured to be incorporated in one of the biochemical steps of lipid digestion processes. For example, prodrugs can be designed to be structurally similar to TG or phospholipids. In such circumstances, prodrugs can be hydrolysed, re-acylated and eventually incorporated with CM during lipoprotein assembly process in the enterocytes [127].

One of the ways to enhance the lipophilicity of a molecule is the synthesis of an ester, ether or amide-linked prodrug with large alkyl moiety [127]. Han et al. [128] described the synthesis of lipophilic prodrugs to promote the delivery of mycophenolic acid (MPA), a model immunomodulator, to the GALT after oral administration with oleic acid. The lipophilicity of MPA (log *P* 2.9) was increased by the synthesis of long-chain ester prodrug (MPA-C18E, log *P* 12.4) and long-chain amide prodrug (MPA-C18AM, log *P* 11.2). Oral administration of MPA-C18E and MPA-C18AM to mesenteric lymph duct-cannulated rats resulted in a 13- and 6-fold increase in lymphatic transport, respectively, compared to the parent compound. This approach enhanced the partitioning of alkyl chain prodrugs to CM and thereby promoted lymphatic transport. However, the least lipophilic medium-chain ester prodrug (MPA-C8E) did not increase the recovery of parent compound in lymph. In the same study, a TG-mimicking prodrug of MPA (2-MPA-TG, log *P* 17.8) was synthesised. The TG-mimicking prodrug leads to an 80-fold increase in lymphatic transport by a mechanism that involves the incorporation of the prodrug in TG

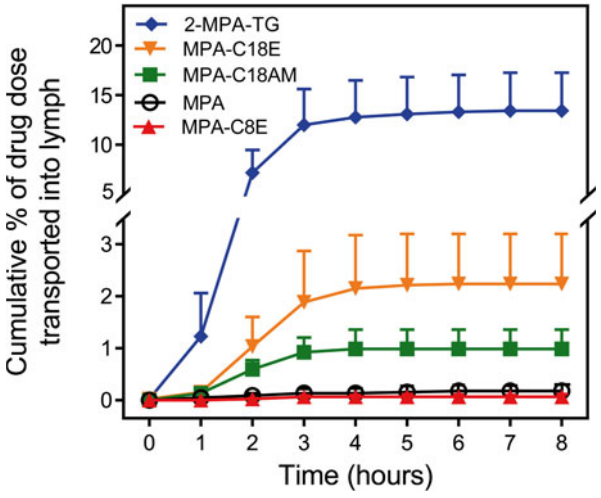

Fig. 14.5 Cumulative intestinal lymphatic transport of mycophenolic acid (MPA, open circles, $n=5$), its medium-chain ester prodrug (MPA-C8E, triangles, $n=3$), long-chain ester prodrug (MPA-C18E, inverted triangles, $n=6$), long-chain amide prodrug (MPA-C18AM, squares, $n=4$) and triglyceride mimic prodrug (2-MPA-TG, diamonds, $n=5$) versus time following intraduodenal administration with oleic acid, Tween 80 and PBS in mesenteric lymph duct-cannulated anaesthetised rats. Data presented as mean ± SD (Reproduced with permission from Han et al. [128])

hydrolysis-reacylation and CM assembly pathway. The authors suggested that metabolic instability and poor absorption were behind low lymphatic concentrations of alkyl prodrugs relative to TG-mimicking prodrug (Fig. 14.5).

Future Directions

Currently, there is a high number of immunomodulators that show remarkable therapeutic benefits in the treatment of life-threatening immune disorders. If delivered to the GALT, these immunomodulators have potential to significantly improve future opportunities to treat these disorders. Examples of such molecules are statins which are widely used in clinical practice as cholesterol-lowering agents. Animal models of autoimmune diseases have shown that statins have therapeutic immunomodulatory effects in the treatment of multiple sclerosis [129–131], rheumatoid arthritis [132, 133], autoimmune myocarditis [134] and autoimmune uveitis [135]. However, doses of statins used in these experiments were higher than those usually used in humans. Thus, using an appropriate strategy to target GALT, statins might achieve sufficient concentrations to produce therapeutic immunomodulatory effects while inducing less systemic adverse effects in off-target tissues. In fact, researchers have used lipid-based formulations like SEDDS [136], SMEDDS [137], SNEDDS [138], SLN [139] and nanostructured lipid carriers (NLC, mentioned below) [140] to improve the oral bioavailability of statins, yet neither intestinal lymphatic transport nor immunomodulatory effects were assessed upon the administration of these formulations. Therefore, it is tempting to suggest that statins might have new therapeutic applications if properly targeted to GALT in patients with autoimmune diseases.

An important group of potential therapeutic immunomodulators are lipophilic cannabinoids, such as Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Pharmacodynamic studies have shown that both cannabinoids have broad spectrum of therapeutic activities [141–144]. Animal models studies of immune system disorders have reported that THC could be a promising drug in the treatment of multiple sclerosis [145], diabetes mellitus [146] and allergic asthma [147]. CBD also showed therapeutic efficacy in animal models of rheumatoid arthritis [148], diabetes mellitus [149] and allergic asthma [147]. Both THC and CBD are highly lipophilic molecules with log $D_{7.4}$ of 7.25 and 6.99, respectively, which makes them good candidates for targeting to the GALT if orally co-administered with LCT.

Additional novel chemical or formulation-based strategies for targeting drugs to GALT could lead to increased targeting efficiency. One worth mentioning is nanostructured lipid carriers (NLC). After the development and success of SLN, a number of problems were identified. Using a single type of solid lipid in the core of SLN led to the formation of a crystalline lattice over time that potentially reduced drug-loading capacity [100]. Gelation of SLN also occurred after prolonged storage [101] and NLC were created as a way to reduce these problems. NLC use a mixture of solid and liquid lipids to create an imperfect core environment so there is more

space to accommodate drugs, while still maintaining a solid state [104, 150]. It can therefore be said that NLC are a second-generation lipid-based nanoparticle formulation [100]. Due to the relatively new development of NLC, there has not been as extensive study into the targeting of NLC to the GALT, but research has begun in assessment for their targeting potential. One work studied the oral administration of NLC loaded with a lipophilic vasodilator [150]. The oral administration of these NLC resulted in a threefold increase in bioavailability of the loaded drug compared to an aqueous suspension. This suggests that NLC activated an alternative absorption pathway, possibly also avoiding first-pass metabolism, a frequent cause of low oral bioavailability for many lipophilic drugs [151]. It is therefore conceivable that immunomodulators can be loaded in NLC and targeted towards the GALT in a similar fashion.

Conclusions

Immunomodulatory drugs have advanced treatment protocols of a wide range of disorders where immune system is actively involved, such as rheumatoid arthritis and multiple sclerosis. However, despite considerable advances, some immunomodulators might cause serious adverse effects, which could be the cause of treatment failure for these therapies [152]. Systemic adverse effects are more prominent in non-targeted administration. Therefore, enhancing the delivery of immunomodulatory drugs to immune cells has potential to reduce systemic adverse effects as well as improve treatment efficacy [18].

Different approaches of targeting GALT by immunomodulatory drugs have successfully increased the concentration of drugs achieved in the GALT and, more importantly, significantly enhanced immunomodulatory effects. Immunomodulatory drugs of diverse physicochemical properties have been targeted to GALT, and this strategy presents a promising new way to treat diseases involving the immune system.

References

1. Loukas M, Bellary SS, Kuklinski M, Ferrauiola J, Yadav A, Shoja MM, et al. The lymphatic system: a historical perspective. Clin Anat. 2011;24(7):807–16. Epub 2011/05/06.
2. Skobe M, Detmar M. Structure, function, and molecular control of the skin lymphatic system. J Investig Dermatol Symp Proc. 2000;5(1):14–9. Epub 2001/01/09.
3. Eales NB. The history of the lymphatic system, with special reference to the Hunter-Monro controversy. J Hist Med Allied Sci. 1974;29(3):280–94. Epub 1974/07/01.
4. Chikly B. Who discovered the lymphatic system. Lymphology. 1997;30(4):186–93. Epub 1998/02/26.
5. Brown P. Lymphatic system: unlocking the drains. Nature. 2005;436(7050):456–8.
6. Swartz MA. The physiology of the lymphatic system. Adv Drug Deliv Rev. 2001;50(1–2):3–20. Epub 2001/08/08.

7. Saroj P, Verma M, Jha K. An overview on immunomodulation. J Adv Sci Res. 2012;3(1):7–12.
8. Patil U, Jaydeokar A, Bandawane D. Immunomodulators: a pharmacological review. Int J Pharm Sci. 2012;4(1):30–6.
9. Masihi KN. Immunomodulatory agents for prophylaxis and therapy of infections. Int J Antimicrob Agents. 2000;14(3):181–91. Epub 2000/04/25.
10. Masihi KN. Immunomodulators in infectious diseases: panoply of possibilites. Int J Immunopharmacol. 2000;22(12):1083–91. Epub 2001/01/04.
11. Saladin KS. Anatomy & physiology: the unity of form and function. New York: McGraw-Hill; 2012.
12. Jurisic G, Detmar M. Lymphatic endothelium in health and disease. Cell Tissue Res. 2009;335(1):97–108. Epub 2008/07/24.
13. Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metab. 2009;296(6):E1183–94. Epub 2009/01/23.
14. Black DD. Development and physiological regulation of intestinal lipid absorption. I. Development of intestinal lipid absorption: cellular events in chylomicron assembly and secretion. Am J Physiol Gastrointest Liver Physiol. 2007;293(3):G519–24. Epub 2007/05/15.
15. Nordskog BK, Phan CT, Nutting DF, Tso P. An examination of the factors affecting intestinal lymphatic transport of dietary lipids. Adv Drug Deliv Rev. 2001;50(1–2):21–44. Epub 2001/08/08.
16. Charman WNA, Stella VJ. Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules. Int J Pharm. 1986;34(1–2):175–8.
17. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. Nat Rev Immunol. 2003;3(11):867–78. Epub 2003/12/12.
18. Trevaskis NL, Charman WN, Porter CJ. Targeted drug delivery to lymphocytes: a route to site-specific immunomodulation? Mol Pharm. 2010;7(6):2297–309. Epub 2010/10/21.
19. Clement C, Santambrogio L. The lymph proteome, peptidome, and degradome. In: Santambrogio L, editor. Immunology of the lymphatic system. New York: Springer; 2013. p. 65–79.
20. Cueni LN, Detmar M. New insights into the molecular control of the lymphatic vascular system and its role in disease. J Invest Dermatol. 2006;126(10):2167–77. Epub 2006/09/20.
21. McAllaster JD, Cohen MS. Role of the lymphatics in cancer metastasis and chemotherapy applications. Adv Drug Deliv Rev. 2011;63(10–11):867–75. Epub 2011/06/28.
22. Shields J. Lymph and lymphatic capillaries in cancer. In: Santambrogio L, editor. Immunology of the lymphatic system. New York: Springer; 2013. p. 121–42.
23. Ohl L, Bernhardt G, Pabst O, Förster R, editors. Chemokines as organizers of primary and secondary lymphoid organs. Semin Immunol. 2003;15(5):249–55. Elsevier.
24. Fu YX, Chaplin DD. Development and maturation of secondary lymphoid tissues. Annu Rev Immunol. 1999;17:399–433. Epub 1999/06/08.
25. Pal I, Ramsey JD. The role of the lymphatic system in vaccine trafficking and immune response. Adv Drug Deliv Rev. 2011;63(10–11):909–22. Epub 2011/06/21.
26. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. Toxicol Pathol. 2006;34(5):599–608. Epub 2006/10/28.
27. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 2003;3(4):331–41. Epub 2003/04/02.
28. Forchielli ML, Walker WA. The role of gut-associated lymphoid tissues and mucosal defence. Br J Nutr. 2005;93 Suppl 1:S41–8. Epub 2005/05/10.
29. Nakajima-Adachi H, Kikuchi A, Fujimura Y, Shibahara K, Makino T, Goseki-Sone M, et al. Peyer's patches and mesenteric lymph nodes cooperatively promote enteropathy in a mouse model of food allergy. PLoS One. 2014;9(10), e107492. Epub 2014/10/08.
30. Wershil BK, Furuta GT. 4. Gastrointestinal mucosal immunity. J Allergy Clin Immunol. 2008;121(2 Suppl):S380–3; quiz S415. Epub 2008/02/05.
31. Brandtzaeg P, Kiyono H, Pabst R, Russell MW. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol. 2008;1(1):31–7. Epub 2008/12/17.

32. Varol C, Zigmond E, Jung S. Securing the immune tightrope: mononuclear phagocytes in the intestinal lamina propria. Nat Rev Immunol. 2010;10(6):415–26. Epub 2010/05/26.
33. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. Nat Rev Immunol. 2011;11(7):445–56. Epub 2011/06/18.
34. Miura S, Sekizuka E, Nagata H, Oshio C, Minamitani H, Suematsu M, et al. Increased lymphocyte transport by lipid absorption in rat mesenteric lymphatics. Am J Physiol. 1987;253(5 Pt 1):G596–600.
35. Hamada H, Hiroi T, Nishiyama Y, Takahashi H, Masunaga Y, Hachimura S, et al. Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. J Immunol. 2002;168(1):57–64. Epub 2001/12/26.
36. Watzl B, Girrbach S, Roller M. Inulin, oligofructose and immunomodulation. Br J Nutr. 2005;93 Suppl 1:S49–55. Epub 2005/05/10.
37. Miura S, Tsuzuki Y, Hokari R, Ishii H. Modulation of intestinal immune system by dietary fat intake: relevance to Crohn's disease. J Gastroenterol Hepatol. 1998;13(12):1183–90. Epub 1999/01/26.
38. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. Nat Rev Immunol. 2008;8(6):435–46. Epub 2008/05/27.
39. Spahn TW, Kucharzik T. Modulating the intestinal immune system: the role of lymphotoxin and GALT organs. Gut. 2004;53(3):456–65. Epub 2004/02/13.
40. Blanchard DK, Budde JM, Hatch 3rd GF, Wertheimer-Hatch L, Hatch KF, Davis GB, et al. Tumors of the small intestine. World J Surg. 2000;24(4):421–9. Epub 2000/03/09.
41. Porter CJ, Charman WN. Intestinal lymphatic drug transport: an update. Adv Drug Deliv Rev. 2001;50(1–2):61–80. Epub 2001/08/08.
42. Trevaskis NL, Charman WN, Porter CJ. Lipid-based delivery systems and intestinal lymphatic drug transport: a mechanistic update. Adv Drug Deliv Rev. 2008;60(6):702–16. Epub 2007/12/25.
43. Porter CJ, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6(3):231–48. Epub 2007/03/03.
44. Wasan KM, Cassidy SM. Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. J Pharm Sci. 1998;87(4):411–24. Epub 1998/04/21.
45. Miura S, Imaeda H, Shiozaki H, Ohkubo N, Tashiro H, Serizawa H, et al. Increased proliferative response of lymphocytes from intestinal lymph during long chain fatty acid absorption. Immunology. 1993;78(1):142–6. Epub 1993/01/01.
46. Calder PC, Yaqoob P, Newsholme EA. Triacylglycerol metabolism by lymphocytes and the effect of triacylglycerols on lymphocyte proliferation. Biochem J. 1994;298(Pt 3):605–11. Epub 1994/03/15.
47. Millar JH, Zilkha KJ, Langman MJ, Wright HP, Smith AD, Belin J, et al. Double-blind trial of linoleate supplementation of the diet in multiple sclerosis. Br Med J. 1973;1(5856):765–8. Epub 1973/03/31.
48. Takagi T, Ramachandran C, Bermejo M, Yamashita S, Yu LX, Amidon GL. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. Mol Pharm. 2006;3(6):631–43. Epub 2006/12/05.
49. Hauss DJ. Oral lipid-based formulations. Adv Drug Deliv Rev. 2007;59(7):667–76. Epub 2007/07/10.
50. Kuentz M. Lipid-based formulations for oral delivery of lipophilic drugs. Drug Discov Today Technol. 2012;9(2):e71–174. Epub 2012/07/01.
51. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. Eur J Pharm Sci. 2000;11 Suppl 2:S93–8. Epub 2000/10/18.
52. Singh BN. Effects of food on clinical pharmacokinetics. Clin Pharmacokinet. 1999;37(3):213–55. Epub 1999/10/08.
53. Armand M, Borel P, Ythier P, Dutot G, Melin C, Senft M, et al. Effects of droplet size, triacylglycerol composition, and calcium on the hydrolysis of complex emulsions by pancreatic lipase: an in vitro study. J Nutr Biochem. 1992;3(7):333–41.
54. Ruf H, Gould BJ. Size distributions of chylomicrons from human lymph from dynamic light scattering measurements. Eur Biophys J EBJ. 1999;28(1):1–11. Epub 1999/02/06.

55. Sarin H. Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. J Angiogenesis Res. 2010;2:14.
56. Noguchi T, Charman WNA, Stella VJ. Lymphatic appearance of DDT in thoracic or mesenteric lymph duct cannulated rats. Int J Pharm. 1985;24(2–3):185–92.
57. Gershkovich P, Fanous J, Qadri B, Yacovan A, Amselem S, Hoffman A. The role of molecular physicochemical properties and apolipoproteins in association of drugs with triglyceride-rich lipoproteins: in-silico prediction of uptake by chylomicrons. J Pharm Pharmacol. 2009;61(1):31–9.
58. Montecucco F, Burger F, Mach F, Steffens S. CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. Am J Physiol Heart Circ Physiol. 2008;294(3):H1145–55. Epub 2008/01/08.
59. Arevalo-Martin A, Vela JM, Molina-Holgado E, Borrell J, Guaza C. Therapeutic action of cannabinoids in a murine model of multiple sclerosis. J Neurosci Off J Soc Neurosci. 2003;23(7):2511–6. Epub 2003/04/10.
60. Klein TW, Cabral GA. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. J Neuroimmun Pharmacol Off J Soc Neuroimmune Pharmacol. 2006; 1(1):50–64. Epub 2007/11/28.
61. Achiron A, Miron S, Lavie V, Margalit R, Biegon A. Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. J Neuroimmunol. 2000;102(1):26–31. Epub 2000/01/08.
62. Gershkovich P, Qadri B, Yacovan A, Amselem S, Hoffman A. Different impacts of intestinal lymphatic transport on the oral bioavailability of structurally similar synthetic lipophilic cannabinoids: dexanabinol and PRS-211,220. Eur J Pharm Sci Off J Eur Federation Pharm Sci. 2007;31(5):298–305. Epub 2007/06/15.
63. Takada K, Yoshimura H, Yoshikawa H, Muranishi S, Yasumura T, Oka T. Enhanced selective lymphatic delivery of cyclosporin A by solubilizers and intensified immunosuppressive activity against mice skin allograft. Pharm Res. 1986;3(1):48–51.
64. Fukui E, Kurohara H, Kageyu A, Kurosaki Y, Nakayama T, Kimura T. Enhancing effect of medium-chain triglycerides on intestinal absorption of d-alpha-tocopherol acetate from lecithin-dispersed preparations in the rat. J Pharmacobiodyn. 1989;12(2):80–6. Epub 1989/02/01.
65. Kovarik JM, Mueller EA, van Bree JB, Tetzloff W, Kutz K. Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. J Pharm Sci. 1994;83(3):444–6. Epub 1994/03/01.
66. Hauss DJ, Fogal SE, Ficorilli JV, Price CA, Roy T, Jayaraj AA, et al. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. J Pharm Sci. 1998;87(2):164–9.
67. Starzl TE, Klintmalm GBG, Porter KA, Iwatsuki S, Schröter GPJ. Liver transplantation with use of cyclosporin A and prednisone. N Engl J Med. 1981;305(5):266–9.
68. Takada K, Furuya Y, Yoshikawa H, Muranishi S. Biological and pharmaceutical factors affecting the absorption and lymphatic delivery of ciclosporin A from gastrointestinal tract. J Pharmacobiodyn. 1988;11(2):80–7. Epub 1988/02/01.
69. Wang CP, Hartman NR, Venkataramanan R, Jardine I, Lin FT, Knapp JE, et al. Isolation of 10 cyclosporine metabolites from human bile. Drug Metab Dispos Biol Fate Chemicals. 1989;17(3):292–6. Epub 1989/05/01.
70. Christians U, Strohmeyer S, Kownatzki R, Schiebel HM, Bleck J, Kohlhaw K, et al. Investigations on the metabolic pathways of cyclosporine: II. Elucidation of the metabolic pathways in vitro by human liver microsomes. Xenobiotica. 1991;21(9):1199–210. Epub 1991/09/01.
71. Tjia JF, Webber IR, Back DJ. Cyclosporin metabolism by the gastrointestinal mucosa. Br J Clin Pharmacol. 1991;31(3):344–6.
72. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Deliv Rev. 2000;45(1):89–121.
73. Choc MG. Bioavailability and pharmacokinetics of cyclosporine formulations: Neoral® vs Sandimmune®. Int J Dermatol. 1997;36:1–6.

74. Zhang Z. Mechanism of enhanced oral absorption of morin by phospholipid complex based self-nanoemulsifying drug delivery system. Mol Pharm. 2015;12(2):504–13.
75. Ahmed K, Li Y, McClements DJ, Xiao H. Nanoemulsion- and emulsion-based delivery systems for curcumin: encapsulation and release properties. Food Chem. 2012;132(2): 799–807.
76. Holm R, Porter CJH, Edwards GA, Müllertz A, Kristensen HG, Charman WN. Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. Eur J Pharm Sci. 2003;20(1):91–7.
77. Lian T, Ho RJY. Trends and developments in liposome drug delivery systems. J Pharm Sci. 2001;90(6):667–80.
78. Gabizon A, Dagan A, Goren D, Barenholz Y, Fuks Z. Liposomes as in vivo carriers of adriamycin: reduced cardiac uptake and preserved antitumor activity in mice. Cancer Res. 1982;42(11):4734–9.
79. Oussoren C, Storm G. Liposomes to target the lymphatics by subcutaneous administration. Adv Drug Deliv Rev. 2001;50(1–2):143–56.
80. Perche F, Torchilin VP. Recent trends in multifunctional liposomal nanocarriers for enhanced tumor targeting. J Drug Deliv. 2013;2013:705265. Epub 2013/03/28.
81. Cho HY, Lee YB. Nano-sized drug delivery systems for lymphatic delivery. J Nanosci Nanotechnol. 2014;14(1):868–80. Epub 2014/04/16.
82. Júnior ÁDC, Mota LG, Nunan EA, Wainstein AJA, Wainstein APDL, Leal AS, et al. Tissue distribution evaluation of stealth pH-sensitive liposomal cisplatin versus free cisplatin in Ehrlich tumor-bearing mice. Life Sci. 2007;80(7):659–64.
83. Leite EA, Giuberti Cdos S, Wainstein AJ, Wainstein AP, Coelho LG, Lana AM, et al. Acute toxicity of long-circulating and pH-sensitive liposomes containing cisplatin in mice after intraperitoneal administration. Life Sci. 2009;84(19–20):641–9. Epub 2009/03/24.
84. Kneidl B, Peller M, Winter G, Lindner LH, Hossann M. Thermosensitive liposomal drug delivery systems: state of the art review. Int J Nanomedicine. 2014;9:4387–98.
85. McCarley RL. Redox-responsive delivery systems. Ann Rev Anal Chem (Palo Alto Calif). 2012;5:391–411. Epub 2012/06/20.
86. Wang C, Liu P, Zhuang Y, Li P, Jiang B, Pan H, et al. Lymphatic-targeted cationic liposomes: a robust vaccine adjuvant for promoting long-term immunological memory. Vaccine. 2014;32(42):5475–83.
87. Eloy JO, Claro de Souza M, Petrilli R, Barcellos JP, Lee RJ, Marchetti JM. Liposomes as carriers of hydrophilic small molecule drugs: strategies to enhance encapsulation and delivery. Colloids Surf B Biointerfaces. 2014;123c:345–63. Epub 2014/10/05.
88. Dromi S, Frenkel V, Luk A, Traughber B, Angstadt M, Bur M, et al. Pulsed-high intensity focused ultrasound and low temperature-sensitive liposomes for enhanced targeted drug delivery and antitumor effect. Clin Cancer Res Off J Am Assoc Cancer Res. 2007;13(9):2722–7. Epub 2007/05/03.
89. Husseini GA, Pitt WG, Martins AM. Ultrasonically triggered drug delivery: breaking the barrier. Colloids Surf B Biointerfaces. 2014;123c:364–86. Epub 2014/12/03.
90. Wang JY, Wu QF, Li JP, Ren QS, Wang YL, Liu XM. Photo-sensitive liposomes: chemistry and application in drug delivery. Mini Rev Med Chem. 2010;10(2):172–81. Epub 2010/04/23.
91. Videira M, Almeida AJ, Fabra À. Preclinical evaluation of a pulmonary delivered paclitaxel-loaded lipid nanocarrier antitumor effect. Nanomedicine Nanotechnol Biol Med. 2012;8(7):1208–15.
92. Barrowman JA. Physiology of the gastro-intestinal lymphatic system. Cambridge: Cambridge University Press; 1978.
93. Perry MA, Granger DN. Permeability of intestinal capillaries to small molecules. Am J Physiol. 1981;241(1):G24–30. Epub 1981/07/01.
94. Perrie Y, Obrenovic M, McCarthy D, Gregoriadis G. Liposome (Lipodine)-mediated DNA vaccination by the oral route. J Liposome Res. 2002;12(1–2):185–97. Epub 2003/02/27.
95. Masuda K, Horie K, Suzuki R, Yoshikawa T, Hirano K. Oral delivery of antigens in liposomes with some lipid compositions modulates oral tolerance to the antigens. Microbiol Immunol. 2002;46(1):55–8. Epub 2002/03/26.

96. Badiee A, Khamesipour A, Samiei A, Soroush D, Shargh VH, Kheiri MT, et al. The role of liposome size on the type of immune response induced in BALB/c mice against leishmaniasis: rgp63 as a model antigen. Exp Parasitol. 2012;132(4):403–9.
97. Nishioka Y, Yoshino H. Lymphatic targeting with nanoparticulate system. Adv Drug Deliv Rev. 2001;47(1):55–64.
98. Eldridge JH, Meulbroek JA, Staas JK, Tice TR, Gilley RM. Vaccine-containing biodegradable microspheres specifically enter the gut-associated lymphoid tissue following oral administration and induce a disseminated mucosal immune response. Adv Exp Med Biol. 1989;251:191–202. Epub 1989/01/01.
99. Florence AT, Hussain N. Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. Adv Drug Deliv Rev. 2001;50(Suppl 1(0)):S69–89.
100. Westesen K, Bunjes H, Koch MHJ. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release. 1997;48(2–3):223–36.
101. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161–77.
102. Bargoni A, Cavalli R, Zara GP, Fundarò A, Caputo O, Gasco MR. Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (sln) after duodenal administration to rats. Part II—tissue distribution. Pharmacol Res. 2001;43(5):497–502.
103. Horne RW, Bangham AD, Whittaker VP. Negatively stained lipoprotein membranes. Nature. 1963;200(4913):1340.
104. Das S, Chaudhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. AAPS PharmSciTech. 2011;12(1):62–76.
105. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov. 2005;4(2):145–60.
106. Yao M, Xiao H, McClements DJ. Delivery of lipophilic bioactives: assembly, disassembly, and reassembly of lipid nanoparticles. Ann Rev Food Sci Technol. 2014;5(1):53–81.
107. Garcia-Fuentes M, Prego C, Torres D, Alonso MJ. A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly(ethylene glycol) as carriers for oral calcitonin delivery. Eur J Pharm Sci. 2005;25(1):133–43. Epub 2005/04/28.
108. Harivardhan Reddy L, Sharma RK, Chuttani K, Mishra AK, Murthy RSR. Influence of administration route on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in Dalton's lymphoma tumor bearing mice. J Control Release. 2005;105(3):185–98.
109. Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J Control Release. 2005;107(2):215–28.
110. Choi SH, Jin S-E, Lee M-K, Lim S-J, Park J-S, Kim B-G, et al. Novel cationic solid lipid nanoparticles enhanced p53 gene transfer to lung cancer cells. Eur J Pharm Biopharm. 2008;68(3):545–54.
111. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, et al. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomedicine Nanotechnol Biol Med. 2009;5(2):184–91.
112. Chalikwar SS, Belgamwar VS, Talele VR, Surana SJ, Patil MU. Formulation and evaluation of Nimodipine-loaded solid lipid nanoparticles delivered via lymphatic transport system. Colloids Surf B Biointerfaces. 2012;97:109–16.
113. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47(2–3):165–96.
114. Ali Khan A, Mudassir J, Mohtar N, Darwis Y. Advanced drug delivery to the lymphatic system: lipid-based nanoformulations. Int J Nanomedicine. 2013;8:2733–44. Epub 2013/08/09.
115. Qi J, Lu Y, Wu W. Absorption, disposition and pharmacokinetics of solid lipid nanoparticles. Curr Drug Metab. 2012;13(4):418–28. Epub 2012/03/27.
116. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. N Engl J Med. 2000;343(22):1594–602. Epub 2000/11/30.

117. Shea B, Swinden MV, Tanjong Ghogomu E, Ortiz Z, Katchamart W, Rader T, et al. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. Cochrane Database Syst Rev. 2013;5:CD000951. Epub 2013/06/04.
118. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci. 2002;6(4):319–27.
119. Zakeri-Milani P, Loveymi BD, Jelvehgari M, Valizadeh H. The characteristics and improved intestinal permeability of vancomycin PLGA-nanoparticles as colloidal drug delivery system. Colloids Surf B Biointerfaces. 2013;103:174–81.
120. Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. J Biomater Sci Polym Ed. 2006;17(3):247–89. Epub 2006/05/13.
121. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly(D, L-lactide-co-glycolide) and its derivatives. J Control Release Off J Control Release Soc. 2008;125(3):193–209. Epub 2007/12/18.
122. Kim W-U, Lee W-K, Ryoo J-W, Kim S-H, Kim J, Youn J, et al. Suppression of collagen-induced arthritis by single administration of poly(lactic-co-glycolic acid) nanoparticles entrapping type II collagen: a novel treatment strategy for induction of oral tolerance. Arthritis Rheum. 2002;46(4):1109–20.
123. De S, Rebouças J, Irache JM, Camacho AI, Gastaminza G, Sanz ML, Ferrer M, et al. Immunogenicity of peanut proteins containing poly(anhydride) nanoparticles. Clin Vaccine Immunol. 2014;21(8):1106–12.
124. Kosaka S, Tamauchi H, Terashima M, Maruyama H, Habu S, Kitasato H. IL-10 controls Th2-type cytokine production and eosinophil infiltration in a mouse model of allergic airway inflammation. Immunobiology. 2011;216(7):811–20.
125. Stella VJ. A case for prodrugs. Prodrugs: Springer New York; 2007. p. 3–33.
126. Gershkovich P, Hoffman A. Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability. Eur J Pharm Sci Off J Eur Federation Pharm Sci. 2005;26(5):394–404. Epub 2005/09/06.
127. Shackleford DM, Porter CJ, Charman WN. Lymphatic absorption of orally administered prodrugs. Prodrugs: Springer New York; 2007. p. 653–82.
128. Han S, Quach T, Hu L, Wahab A, Charman WN, Stella VJ, et al. Targeted delivery of a model immunomodulator to the lymphatic system: comparison of alkyl ester versus triglyceride mimetic lipid prodrug strategies. J Control Release Off J Control Release Soc. 2014;177:1–10. Epub 2014/01/09.
129. Nath N, Giri S, Prasad R, Singh AK, Singh I. Potential targets of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor for multiple sclerosis therapy. J Immunol. 2004;172(2):1273–86. Epub 2004/01/07.
130. Youssef S, Stuve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature. 2002;420(6911):78–84. Epub 2002/11/08.
131. Stanislaus R, Singh AK, Singh I. Lovastatin treatment decreases mononuclear cell infiltration into the CNS of Lewis rats with experimental allergic encephalomyelitis. J Neurosci Res. 2001;66(2):155–62.
132. Leung BP, Sattar N, Crilly A, Prach M, McCarey DW, Payne H, et al. A novel anti-inflammatory role for simvastatin in inflammatory arthritis. J Immunol. 2003;170(3):1524–30.
133. Barsante MM, Roffê E, Yokoro CM, Tafuri WL, Souza DG, Pinho V, et al. Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis. Eur J Pharmacol. 2005;516(3):282–9.
134. Liu W, Li W-M, Gao C, Sun N-L. Effects of atorvastatin on the Th1/Th2 polarization of ongoing experimental autoimmune myocarditis in Lewis rats. J Autoimmun. 2005;25(4):258–63.

135. Gegg ME, Harry R, Hankey D, Zambarakji H, Pryce G, Baker D, et al. Suppression of autoimmune retinal disease by lovastatin does not require Th2 cytokine induction. J Immunol. 2005;174(4):2327–35.
136. Patil P, Patil V, Paradkar A. Formulation of a self-emulsifying system for oral delivery of simvastatin: in vitro and in vivo evaluation. Acta Pharm. 2007;57(1):111–22. Epub 2007/03/01.
137. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, et al. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm. 2004;274(1–2):65–73. Epub 2004/04/10.
138. Mahmoud H, Al-Suwayeh S, Elkadi S. Design and optimization of self-nanoemulsifying drug delivery systems of simvastatin aiming dissolution enhancement. Afr J Pharm Pharmacol. 2013;7(22):1482–500.
139. Padhye SG, Nagarsenker MS. Simvastatin solid lipid nanoparticles for oral delivery: formulation development and in vivo evaluation. Indian J Pharm Sci. 2013;75(5):591–8. Epub 2014/01/10.
140. Tiwari R, Pathak K. Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: comparative analysis of characteristics, pharmacokinetics and tissue uptake. Int J Pharm. 2011;415(1–2):232–43. Epub 2011/06/07.
141. Zhornitsky S, Potvin S. Cannabidiol in humans-the quest for therapeutic targets. Pharm (Basel). 2012;5(5):529–52. Epub 2012/01/01.
142. Pertwee RG. Pharmacological and therapeutic targets for Δ9 tetrahydrocannabinol and cannabidiol. Euphytica. 2004;140(1–2):73–82.
143. Ben AM. Cannabinoids in medicine: a review of their therapeutic potential. J Ethnopharmacol. 2006;105(1–2):1–25.
144. Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview. Immunobiology. 2010;215(8):588–97.
145. Lyman W, Sonett J, Brosnan C, Elkin R, Bornstein M. Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. J Neuroimmunol. 1989; 23(1):73–81.
146. Li X, Kaminski NE, Fischer LJ. Examination of the immunosuppressive effect of delta9-tetrahydrocannabinol in streptozotocin-induced autoimmune diabetes. Int Immunopharmacol. 2001;1(4):699–712. Epub 2001/05/19.
147. Jan TR, Farraj AK, Harkema JR, Kaminski NE. Attenuation of the ovalbumin-induced allergic airway response by cannabinoid treatment in A/J mice. Toxicol Appl Pharmacol. 2003;188(1):24–35. Epub 2003/04/02.
148. Malfait A, Gallily R, Sumariwalla P, Malik A, Andreakos E, Mechoulam R, et al. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci U S A. 2000;97(17):9561–6.
149. Weiss L, Zeira M, Reich S, Slavin S, Raz I, Mechoulam R, et al. Cannabidiol arrests onset of autoimmune diabetes in NOD mice. Neuropharmacology. 2008;54(1):244–9. Epub 2007/08/24.
150. Zhuang C-Y, Li N, Wang M, Zhang X-N, Pan W-S, Peng J-J, et al. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int J Pharm. 2010;394(1–2):179–85.
151. Szakacs T, Veres Z, Vereczkey L. In vitro-in vivo correlation of the pharmacokinetics of vinpocetine. Pol J Pharmacol. 2001;53(6):623–8. Epub 2002/05/03.
152. Sathish JG, Sethu S, Bielsky MC, de Haan L, French NS, Govindappa K, et al. Challenges and approaches for the development of safer immunomodulatory biologics. Nat Rev Drug Discov. 2013;12(4):306–24. Epub 2013/03/29.

Chapter 15
The Neuroimmunology of Gluten Intolerance

Marios Hadjivassiliou, David S. Sanders, and Daniel Aeschlimann

Abstract The term gluten-related disorders (GRD) denotes a spectrum of diverse immune-mediated diseases triggered by the ingestion of gluten (protein found in wheat, barley and rye). Coeliac disease (CD) or gluten-sensitive enteropathy is the most recognised and studied entity within GRD. Extraintestinal manifestations are gaining recognition and are increasingly the subject of further studies as they may hold the key to unravelling the pathophysiology of GRD. Such manifestations include skin involvement in the form of dermatitis herpetiformis (DH) and neurological dysfunction (e.g. gluten ataxia and gluten neuropathy). Furthermore, the recent concept of extraintestinal manifestations without enteropathy (termed non-coeliac gluten sensitivity (NCGS)) has become accepted as part of the same spectrum. In this chapter, we review the neurological manifestations in GRD and discuss recent advances in diagnosis and possible pathophysiological mechanisms.

Keywords Coeliac disease • Gluten sensitivity • Gluten ataxia • Gluten neuropathy • Neurological manifestations • Transglutaminase antibodies • Immune pathogenesis • Gluten-sensitive enteropathy

Introduction

Coeliac disease (CD) was first described by the Greek doctor Aretaeus the Cappadocian, in 100 AD. Long time after, Samuel Gee published his lecture [37] "on the coeliac affection" in which he described the classic presentation of the

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disease in children. The aetiological agent remained obscure until the observations of Willem Dicke, a Dutch paediatrician, in 1953 of "the presence in wheat, of a factor having a deleterious effect in cases of coeliac disease" [30]. The introduction of endoscopy and small bowel biopsy in the 1950s confirmed the bowel as the principal organ involved [104]. Such biopsies demonstrated for the first time the typical histological abnormalities that now define gluten-sensitive enteropathy: villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes.

The first evidence of gluten sensitivity-related extraintestinal manifestations became apparent in 1963 when a group of dermatologists published the interesting observation that dermatitis herpetiformis, an itchy vesicular rash, was in fact a form of gluten-related dermatopathy sharing the same small bowel pathology, but less prominent or even no gastrointestinal symptoms [95]. The only reason why small bowel biopsies were done in this group of patients was the observation of persistently low albumin suggestive of protein loss from the gut.

A small number of case reports of patients with presumed CD and neurological manifestations [33, 105, 133] were published prior to the discovery of the aetiological agent and the introduction of small bowel biopsy. Such reports need to be treated with caution given that a diagnosis of CD in those patients was speculative.

The first comprehensive case series of neurological manifestations in the context of histologically confirmed CD was published in 1966 [24]. This detailed clinical and pathology work described the range of neurological manifestations seen in 16 patients with established CD. Of interest was the fact that all patients had gait ataxia and some had peripheral neuropathy as well. The assumption was that such manifestations were nutritional. Indeed all of these patients were grossly malnourished and cachectic. Post-mortem data, however, demonstrated an inflammatory process primarily affecting the cerebellum, but also involving other parts of the central and peripheral nervous systems, a finding that was in favour of an immune-mediated pathogenesis.

Single and multiple case reports of patients with established CD who then developed neurological dysfunction continued to be published [8, 9, 13, 21, 22, 29, 34, 64, 76, 79, 81, 83, 87, 98, 100, 126, 132].

Key findings from these reports were that ataxia and neuropathy were the commonest manifestations always seen in the context of established CD and almost always attributed to nutritional deficiencies. Some reports reported improvement of the neurological problems with adherence to a GFD whilst others did not. None of these reports however documented the strictness of adherence to the gluten-free diet by regular serological testing.

Thirty years after the first comprehensive case series on neurological manifestations of CD saw the publication of a study [41] approaching the subject purely from a neurological perspective. This study investigated the prevalence of serological markers of gluten sensitivity (at the time, IgG and IgA antigliadin antibodies) in patients presenting with neurological dysfunction. The results demonstrated significantly higher prevalence of antigliadin antibodies (AGA) in the neurology group of patients with unclear diagnosis when compared to healthy blood donors and patients with a clear neurological diagnosis. Based on duodenal biopsies, the study showed that the

prevalence of CD was 16 times higher than the prevalence of CD in the healthy population. This study sparked an interest for both neurologists and gastroenterologists in a possible link between sensitivity to gluten and neurological disease.

Epidemiology of Neurological Manifestations

There are now several epidemiological studies from Europe and America and a few from other continents demonstrating that the prevalence of CD in healthy individuals is on the increase [17]. Thus, the prevalence of CD in the healthy population has been shown to be at least 1 % [115]. There are no accurate figures of the prevalence of the neurological manifestations of gluten sensitivity in the general population. Some studies have concentrated on the prevalence of neurological dysfunction amongst patients with established CD. Figures of between 10 % and 22.5 % have been published [12, 65]. Such figures are unlikely to be accurate because they are retrospective, derived solely from gastrointestinal clinics and concentrated exclusively on patients with the classic (i.e. gastrointestinal) CD presentation. Some of these studies also included neurological diseases that are highly unlikely to be gluten related (e.g. carpal tunnel syndrome, idiopathic Parkinson's disease).

Some estimates of prevalence can be made from patient populations attending specialist clinics although caution must be exercised in extrapolating these as they are inevitably affected by referral bias. For example, data collected from the Sheffield dedicated CD clinic (the biggest in the UK) and from the dedicated gluten sensitivity/neurology clinic (the only one in the UK) suggested that for every 7 patients presenting to the gastroenterologist who are then diagnosed with CD, 2 patients present to the neurologist with neurological dysfunction leading to the diagnosis of CD [55]. This ratio is likely to be an underestimate because it does not take into account those patients with neurological manifestations due to sensitivity to gluten that do not have enteropathy (NCGS). In fact, approximately two thirds of patients presenting with neurological dysfunction do not have enteropathy on duodenal biopsy. The authors believe that the prevalence of neurological dysfunction even in patients with CD presenting to gastroenterologists is likely to be much higher to what has been published if patients undergo rigorous neurological workup including MR spectroscopy of the cerebellum. Preliminary results from a prospective study using patients with newly diagnosed CD presenting to a gastroenterologist demonstrated that up to 50 % of such patients have abnormal MR spectroscopy (low NAA/Cr ratios) of the cerebellum [59, 60]. One study in patients with established CD has shown such abnormalities in up to 80 % of patients [58], whilst another study has shown that the prevalence of peripheral neuropathy in this group of patients was 23 % [92]. The above figures are based on patients with CD. The frequency of neurological dysfunction in patients with NCGS is not known. Judging by the fact that two thirds of the cohort of patients seen and assessed in a dedicated gluten/neurology clinic, Sheffield, UK, have NCGS, it is likely that the prevalence of neurological cases with NCGS is even higher than those with CD.

Diagnosis of the Spectrum of Gluten-Related Diseases

The diagnosis of CD in the hands of an experienced gastroenterologist and gastrointestinal histopathologist can be relatively straightforward. CD is after all defined by the presence of an enteropathy (triad of villus atrophy, crypt hyperplasia and increased intraepithelial lymphocytes), usually a reliable gold standard. It is now, however, accepted that the presence of enteropathy is not a prerequisite for the diagnosis of GRD particularly for those patients where neurological or other extraintestinal manifestations are the presenting feature. Furthermore, the triad of the small bowel mucosal changes mentioned above are merely one part of the small bowel histological spectrum (Marsh classification) that ranges from a normal mucosa to a pre-lymphomatous state [94]. Given that the bowel histology in some cases (as per Marsh classification) can be normal, trying to define GRD using solely the bowel biopsies becomes problematic. Whilst serological testing has enhanced the ability to identify patients with enteropathy, none of these tests are 100 % specific or sensitive. For example, endomysial antibody (EMA) and anti-transglutaminase-2 (TG2) IgA antibody detection are specific for the presence of an enteropathy. These markers are frequently negative in patients with neurological or other extraintestinal manifestations who do not have an enteropathy [55].

The majority of patients presenting with neurological manifestations have no gastrointestinal symptoms [55]. Even patients with CD may not have gastrointestinal symptoms. In patients without overt gastrointestinal involvement (enteropathy), serum antibodies to TG2 may be absent [78]. Patients with extraintestinal manifestations typically have antibodies primarily reacting with different TG isozymes, TG3 in DH and TG6 in patients with gluten ataxia [52, 53, 116]. Reaction of such antibodies with TG2 that takes the form of IgA deposits against TG2 in the intestinal mucosa occurs prior to overt changes in small intestinal morphology and sometimes even before the antibodies are detectable in serum [84]. Such antibody deposits seem to be present in patients with neurological and other extraintestinal manifestations as well and may therefore be diagnostically useful [48]. However, this test is not readily available and requires experience in its interpretation. In practice for suspected neurological manifestations, it is best to perform serological tests for both IgA and IgG antibodies to TG2 (and if available anti-TG6 and anti-TG3) and IgG and IgA antibodies to gliadin. Endomysium antibodies are very specific for the detection of enteropathy, but they detect the same antigen (transglutaminase 2) and have thus largely been replaced by TG2 antibody testing. Any differences between the 2 tests however are likely to be related to the different methodologies used (ELISA for TG2 vs immunofluorescence for the detection of EMA). The diagnosis of NCGS remains problematic by the absence of any biological markers. At the moment, such diagnosis is based on symptomatic improvement after the introduction of a GFD and recurrence of symptoms on reintroduction of gluten in the diet. Antigliadin antibodies of the IgG type can be present in up to 25 % of patients with NCGS attending gastroenterology clinics, and such patients may also have increased intraepithelial lymphocytes [130].

CD has a strong genetic predisposition whereby ~40 % of the genetic load comes from MHC class II association [67]. In Caucasian populations, more than 90 % of CD patients carry the HLA DQ2, with the remaining having the HLA DQ8. A small number of CD patients do not belong into either of these groups, but these have been shown to carry just one chain of the DQ2 heterodimer [71]. HLA genetic testing is therefore another useful tool, particularly as unlike other serological tests it is not dependent on an immunological trigger. However, the HLA DQ genotype can be used only as a test of exclusion of CD as the risk genotype DQ2 is common in Caucasian and Asian populations and many carriers will never develop GRD. Furthermore, patients with NCGS may not have the HLA DQ2 or DQ8. Several genome-wide association studies over the past decade have identified many non-HLA loci that each contribute a small amount of risk for coeliac disease [90]. Most of these additional genes are involved in immune functions, and in fact, several risk loci are shared with other autoimmune conditions including ankylosing spondylitis, rheumatoid arthritis, type 1 diabetes and psoriasis. A recent study showed that including non-HLA variants in addition to HLA in the test for coeliac disease risk improves the accuracy of disease prediction [112]. A bias for loci conferring risk for specific manifestations of GRD remains to be thoroughly investigated.

The Spectrum of Gluten-Related Neurological Manifestations

Gluten Ataxia

Gluten ataxia (GA) is defined as idiopathic sporadic ataxia with positive serological markers for sensitivity to gluten [47]. The original definition was based on the serological tests available at the time (antigliadin IgG and IgA antibodies). In a series of 1000 patients with progressive ataxia evaluated over a period of 20 years in Sheffield, UK, GA had a prevalence of 15 % amongst all ataxias but as high as 41 % amongst idiopathic sporadic ataxias. Using the same AGA assay, the prevalence of positive AGA in genetically confirmed ataxias was 14/110 (13 %) and in healthy volunteers 149/1200 (12 %). A number of studies looking at the prevalence of antigliadin antibodies in ataxias have been published [1, 2, 6, 14–16, 23, 41, 46, 47, 69, 91, 106]. The variations in prevalence may relate to geographical differences in the prevalence of CD, referral bias, variability in the AGA assays used, patient selection (some studies included as idiopathic sporadic ataxia patients with cerebellar variant of multisystem atrophy), small number of patients studied and no controls. The common theme in the majority of these studies is the consistently high prevalence of AGA antibodies in sporadic ataxias when compared to healthy controls.

GA usually presents with pure cerebellar ataxia or rarely ataxia in combination with myoclonus (see below), palatal tremor [52, 53], opsoclonus [26] or, rarely,

chorea [109]. GA is usually of insidious onset with a mean age at onset of 53 years. Rarely the ataxia can be rapidly progressive mimicking paraneoplastic cerebellar degeneration. Gaze-evoked nystagmus and other ocular signs of cerebellar dysfunction are common (80 % of cases). All patients have gait ataxia and the majority have limb ataxia. Less than 10 % of patients with GA will have any gastrointestinal symptoms but 40 % will have evidence of enteropathy on biopsy.

Serological diagnosis still relies on the presence of IgG and/or IgA antigliadin antibodies, but more specific biomarkers have been identified. TG6 antibodies have been found to be present in 73 % of patients with idiopathic sporadic ataxia with positive AGA [52, 53]. Furthermore, 32 % of patients with idiopathic sporadic ataxia negative for other serological markers of sensitivity to gluten were found to be positive for TG6 [54, 60]. This may suggest that the prevalence of gluten ataxia may even be higher than previously thought.

Patients with GA usually have evidence of cerebellar atrophy on MR imaging with particular predilection for the cerebellar vermis. MR spectroscopy of the vermis is abnormal in all patients with GA (low N-acetyl aspartate/creatine ratio) with less prominent changes in the cerebellar hemispheres. Even in patients with GA without cerebellar atrophy, MR spectroscopy is abnormal. MR spectroscopy is a useful monitoring tool. Patients who adhere to strict gluten-free diet often have evidence of improvement of the NAA/Cr ratio within the vermis after a year on the diet.

The response to treatment with a gluten-free diet depends on the duration of the ataxia prior to the diagnosis of sensitivity to gluten. Loss of Purkinje cells in the cerebellum, the end result of prolonged gluten exposure in patients with GA, is irreversible; therefore, prompt treatment is more likely to result in improvement or stabilisation of the ataxia. Whilst the benefits of a gluten-free diet in the treatment of patients with CD and DH have long been established, there are very few studies, mainly case reports, of the effect of gluten-free diet on the ataxia. Most of these reports primarily concern patients with established CD who then develop ataxia [7, 61, 106, 107]. These reports suggest overall favourable responsiveness to a gluten-free diet. A small, uncontrolled study and another case study looked at the use of intravenous immunoglobulins in the treatment of patients with GA with and without enteropathy ([14, 15, 114]). All patients improved. In all of these reports, strict adherence to the gluten-free diet was assumed and no serological evidence was provided. The best marker of strict adherence to a gluten-free diet is serological evidence of elimination of gluten-related antibodies. Only one systematic study of the effect of gluten-free diet on a cohort of patients presenting with ataxia, with or without an enteropathy, has been published [46, 47]. This study also reported serological evidence of elimination of the antigliadin antibodies as a confirmation of strict adherence to the diet. Forty-three patients with gluten ataxia were enrolled. Twenty-six adhered strictly to the gluten-free diet, had serological evidence of elimination of antibodies and comprised the treatment group. Fourteen patients refused the diet and comprised the control group. Patient and control groups were matched at baseline for all variables (age, duration of ataxia, etc.). There was no significant difference in the baseline performance for each ataxia test between the two groups. There was significant improvement in performance in test scores and in the subjective

global clinical impression scale in the treatment group when compared to the control group. The improvement was apparent even after excluding patients with an enteropathy. The study concluded that gluten-free diet is an effective treatment for GA.

The current recommendation is that patients presenting with idiopathic progressive cerebellar ataxia should be screened for sensitivity to gluten using antigliadin IgG and IgA, anti-TG2, anti-TG6 and endomysium antibodies [59, 60]. Patients positive for any of these antibodies with no alternative cause for their ataxia should be offered a strict gluten-free diet with regular follow-up to ensure that the antibodies are eliminated (usually takes 6 to 12 months). Stabilisation or even improvement of the ataxia at 1 year would be a strong indicator that the patient suffers from gluten ataxia. The commonest reason for lack of response is compliance with the diet. If patients on strict gluten-free diet continue to progress, with or without elimination of antibodies, the use of immunosuppressive medication (mycophenolate) should be considered. Such cases are rare.

Myoclonic Ataxia and Refractory Coeliac Disease

In 1986, Lu and colleagues published two cases with action myoclonus, ataxia and CD who in addition had epilepsy [87]. The authors provided electrophysiological evidence for the cortical origin of the myoclonus. Similar findings of action and stimulus-sensitive cortical myoclonus were subsequently reported in another patient [126]. This patient had cortical reflex and action myoclonus resembling epilepsia partialis continua, with constant arrhythmic myoclonic activity in the right hypothenar muscles. Electrophysiology confirmed the cortical origin of the myoclonus.

A case series was published in 1995 reporting 4 patients with myoclonus and ataxia with electrophysiological evidence of stimulus-sensitive myoclonus of cortical origin [8]. Pathology showed atrophy of the cerebellar hemispheres with Purkinje cell loss. CD was diagnosed in all four, preceding the onset of the neurological manifestations by years. Such patients unlike those with gluten ataxia appear to be poorly responsive to gluten-free diet and follow a progressive course. The largest series published so far reported 9 patients (6 male, 3 female) with ataxia and asymmetrical irregular jerking [117]. The jerking affected one or more limbs and sometimes face, and it was often stimulus sensitive. All patients later developed more widespread jerking. Six patients had a history of Jacksonian march and five had at least one secondarily generalised seizure. Electrophysiology showed evidence of cortical myoclonus. Four had a phenotype of epilepsia partialis continua. There was clinical, imaging and/or pathological evidence of cerebellar involvement in all cases. Eight patients adhered to a strict gluten-free diet with elimination of gluten-related antibodies, despite which there was still evidence of enteropathy in all thus suggestive of refractory coeliac disease. One patient only just started the diet, and 2 died from enteropathy-associated lymphoma. Five patients were treated with mycophenolate and one in addition with rituximab and IV immunoglobulins. Whilst their ataxia and enteropathy improved, the myoclonus remained the most disabling

feature of their illness. This was the first report to highlight the strong association of this unusual phenotype with refractory CD and in 2 of the cases enteropathy-associated lymphoma.

Gluten Neuropathy

Up to 23 % of patients with established CD on gluten-free diet have neurophysiological evidence of a peripheral neuropathy [92]. A large population-based study of over 84,000 patients with CD in Sweden found that CD was associated with polyneuropathy with a hazard ratio of 3.4 [88]. In a UK-based study, 34 % of patients with otherwise idiopathic sporadic sensorimotor axonal length-dependent neuropathy were found to have circulating AGA [48–50]. Using anti-TG2 antibody, an Italian study also found 21 % of patients with peripheral neuropathy to be positive [96]. Finally, in a tertiary referral centre in the USA, retrospective evaluation of patients with neuropathy showed the prevalence of CD to be between 2.5 and 8 % as compared to 1 % in the healthy population [19].

Gluten neuropathy is defined as otherwise idiopathic sporadic neuropathy with serological evidence of sensitivity to gluten. The commonest types are symmetrical sensorimotor axonal length-dependent peripheral neuropathy and sensory ganglionopathy [56]. Other types of neuropathies have also been reported including asymmetrical neuropathy [20, 42, 77], small fibre neuropathy [11] and rarely pure motor neuropathy [42] or autonomic neuropathy [38]. Gluten neuropathy is slowly progressive with a mean age at onset of the neuropathy being 55 years (range 24 to 77) and a mean duration of 9 years (range 1 to 33). A third of the patients will have evidence of enteropathy on biopsy, but the presence or absence of an enteropathy does not influence the effect of a gluten-free diet [48–50].

Limited pathological data available from post-mortem examinations and nerve biopsies are consistent with an inflammatory aetiology (perivascular lymphocytic infiltration). Gluten-free diet has been shown to be beneficial in single and multiple case reports. The only systematic, controlled study of the effect of a gluten-free diet on 35 patients with gluten neuropathy, with regular serological monitoring of the adherence to the gluten-free diet, found significant improvement in the treated compared with the control group after 1 year on gluten-free diet [48–50]. There was significant increase in the sural sensory action potential, the predefined primary endpoint, in the treatment group as well as subjective improvement of the neuropathic symptoms. Subgroup analysis showed that the capacity for recovery is less when the neuropathy is severe.

Sensory ganglionopathy (sometimes also called neuronopathy) is an asymmetric form of pure sensory neuropathy where the pathology is within the dorsal root ganglia. It can be a paraneoplastic syndrome or related to Sjogren's syndrome. It can also be seen in some inherited neurological illnesses such as Friedreich's ataxia and mitochondrial diseases (POLG-1). Sensitivity to gluten has proven to be the

commonest cause of sensory ganglionopathy (ref). In such cases, there is evidence of inflammatory infiltrates within the dorsal root ganglia. The disease progresses slowly if untreated, but strict adherence to a gluten-free diet may result in stabilisation or even improvement of the neuropathy irrespective of the presence of enteropathy [56].

Headache and Gluten Sensitivity (Gluten Encephalopathy)

Headache is a common feature in patients with GRD. The association was first reported in 2001 based in a series of 10 patients with GRD and headache who in addition had CNS white matter abnormalities on MRI scan [44]. The term "gluten encephalopathy" was used to describe them. The headaches are usually episodic and intractable. They can mimic migraines but do not respond to the usual migraine medication. They characteristically resolve with the introduction of a gluten-free diet. Some patients report a very strong association with ingestion of gluten. The white matter abnormalities are not always present but can be diffuse or focal. They do not always resolve following a gluten-free diet. The diet simply arrests progression of these changes, but the white matter changes can be progressive if the patient does not adhere to a strict gluten-free diet. Their distribution is more suggestive of a vascular rather than demyelinating aetiology. In a prospective study of patients, newly diagnosed with CD frequency of intractable headaches was 44 % [59, 60].

In patients with migraine, there is an overrepresentation of CD with a prevalence of 4.4 % vs 0.4 % in the control population [36]. Using PET brain imaging, a study on regional cerebral perfusion demonstrated that 73 % of patients with CD not on a gluten-free diet had at least one hypoperfused brain region as compared to 7 % in healthy controls and in patients with CD on a gluten-free diet [3]. Another study investigated the prevalence of white matter abnormalities in children with CD and found that 20 % of patients had such abnormalities [80].

Over the last 20 years, we have encountered 100 patients with gluten encephalopathy, a figure that includes the initial 10 patients reported in the 2001 series. Gluten encephalopathy does not always occur in isolation, and such patients will often have additional neurological features such as ataxia. A study from the Mayo clinic emphasised the significant cognitive deficits encountered in 13 such patients [66]. By comparison to gluten ataxia and gluten neuropathy, there is a higher prevalence of enteropathy in patients with gluten encephalopathy (59/100), but the age at onset is the same. The observed improvement of the headaches and arrest of progression in the MRI brain abnormalities suggest a causal link with gluten ingestion [119]. Gluten encephalopathy represents a spectrum of clinical presentations with episodic headaches responsive to a gluten-free diet at one end, to severe debilitating headaches sometimes associated with focal neurological deficits. MRI findings range from normal to extensive white matter abnormalities.

Epilepsy

A link between epilepsy and CD was proposed as far back as 1978 [18, 25, 35]. Whilst studies examining the prevalence of CD amongst patients with epilepsy have suggested a prevalence of 1.2–2.3 %, others failed to demonstrate an increased prevalence [111]. A more recent large (28,885 subjects with CD) population-based cohort study showed that patients with CD were at an increased risk of future epilepsy (HR = 1.42). The absolute risk of future epilepsy in patients with CD was 92/100,000 person-years which equates to an excess risk of 27/100,000 person-years [89]. Most studies on the subject suffer from the same methodological problem of treating epilepsy as a homogeneous disorder. The only study that attempted to look at the prevalence of GRD in well-characterised subgroups of patients with epilepsy found a significant association between AGA and temporal lobe epilepsy with hippocampal sclerosis [102]. Of interest are some case reports on patients with CD and epilepsy whose epilepsy improves following the introduction of gluten-free diet [62, 97].

There is a particular form of focal epilepsy associated with occipital calcifications that appears to have a strong link with CD [39]. This entity is common in Italy but rare in other countries. It tends to affect young patients (mean age 16 years), and in the majority, the seizures are resistant to antiepileptic drugs. The pathogenesis of the cerebral calcifications remains unclear. An autopsy study showed that these depositions consisted of both calcium and silica and microscopically were found in three main types: psammoma-like bodies without any identifiable relationship to cells, vessels or other structures; small granular deposition along small vessels; and focal scanty areas of calcium within neurons [127]. As most of the reported cases are from Italy, Spain and Argentina, it has been hypothesised that the syndrome of coeliac disease, epilepsy and cerebral calcifications is “a genetic, non-inherited, ethnically and geographically restricted syndrome associated with environmental factors” [40]. A case study of a 4-year-old boy with refractory epilepsy, occipital calcifications and coeliac disease reported positive antibody binding to neurons and glia using indirect immunofluorescence. High levels of TG6 antibodies were found in the patient’s serum. After the introduction of gluten-free diet, the child became seizure-free [74].

Myopathy

This is a relatively rare neurological manifestation of GRD, first described by Henriksson et al. [63]. This study from Sweden reported that out of 76 patients with suspected polymyositis investigated at a neuromuscular unit, 17 had a history of gastrointestinal symptoms with evidence of malabsorption. Fourteen of these fulfilled the diagnostic criteria for polymyositis and of those 5 were diagnosed with CD. A more recent study from Spain [118] demonstrated the prevalence of AGA

antibodies amongst patients with inflammatory myopathies to be 31 %. This was accompanied by a higher prevalence of CD within the same population when compared to healthy controls.

A case series of 19 patients are based on what we have encountered in the gluten neurology clinic, Sheffield, UK, over the last 20 years. Thirteen of these patients have been reported previously [51]. Enteropathy was identified following duodenal biopsy in 11 of these patients. The mean age at onset of the myopathic symptoms was 54 years. Ten patients had predominantly proximal weakness, 6 patients had both proximal and distal weakness and 4 patients had primarily distal weakness. Two patients had ataxia and neuropathy, and one patient had just neuropathy in addition to the myopathy. Serum creatine kinase (CK) level ranged between normal and 4380 IU/L at presentation (normal, 25–190 IU/L). Inflammatory myopathy was the commonest finding on neuropathological examination. Six patients received immunosuppressive treatment in addition to starting a gluten-free diet, whereas the others went on a gluten-free diet only. In the majority of those patients who did not receive immunosuppressive treatment, there was clinical improvement of the myopathy with gluten-free diet, suggesting that the myopathy was aetiologically linked to the GRD. One patient developed a profound myopathy after inadvertently eating rye flour. He made a full recovery by re-establishing a strict gluten-free diet. Two patients had histological evidence of inclusion body myositis. It is interesting to note that inclusion body myositis shares the same HLA genetic predisposition with CD. One patient was known to have CD already when he developed the myopathy. He was on gluten-free diet already with negative serology for CD. Muscle biopsy showed an inflammatory myopathy, and repeat duodenal biopsy showed a flat mucosa. Further immunohistological examination of the biopsy did not suggest refractory CD. The patient admitted the occasional dietary indiscretion. He underwent further dietary review and has been started on steroids with some clinical improvement.

Myelopathy

Clinical evidence of a myelopathy in the absence of vitamin B12 and other deficiencies (particularly copper) can be a rare manifestation of CD. It is usually associated with normal imaging of the spinal cord although cases of transverse myelitis like MR appearances have been encountered in our cohort of patients. The neurological presentation often coincides with the diagnosis of CD. There have been some case reports of patients with neuromyelitis optica and sensitivity to gluten who have antibodies to aquaporin-4 [72, 73]. Such patients clearly have abnormal MRI of the spinal cord, but the diagnosis of CD was only made at the time of their neurological presentation. Neuromyelitis optica and CD share the same HLA genetic susceptibility (HLA DQ2). There is very limited data on the effect of the diet on the likelihood of relapse of the disease particularly given the fact that most patients with neuromyelitis optica end up on long-term immunosuppressive medication.

Stiff-Man Syndrome

Stiff-man syndrome (SMS) is a rare autoimmune disease characterised by axial stiffness, painful spasms and positivity for anti-GAD. It has a strong association with other autoimmune diseases (e.g. IDDM, hypothyroidism). We have found a high prevalence of gluten-related antibodies in patients with this condition over and above that expected from an association of 2 autoimmune diseases. The relapsing remitting nature of the condition makes a study of any responsiveness to gluten-free diet difficult. There is however evidence of reduction of the anti-GAD antibody titre following the introduction of a gluten-free diet suggesting that the diet may be beneficial in treating the condition [57]. This finding also supports the concept of prevention of autoimmunity in patients with GRD if the gluten-free diet is introduced early enough [129].

The concept of hyperexcitability of the central nervous system in the context of CD is of interest. We have already discussed above the entity of cortical myoclonus and refractory CD and the association with SPS. We have encountered patients with other hyperexcitable CNS disorders such as progressive encephalomyelitis with rigidity and spasms and patients with startle myoclonus who also have CD. A recent study from Italy has demonstrated that a group of 20 patients with newly diagnosed CD (no neurological complaints) had significantly shorter cortical silent period, reduced intracortical inhibition and enhanced intracortical facilitation by comparison to 20 age-matched healthy controls. The authors concluded that a pattern of cortical excitability was found in patients with CD and that immune system dysregulation may be responsible for this [108].

Pathogenesis

Post-mortem data from patients with gluten ataxia demonstrate patchy loss of Purkinje cells throughout the cerebellar cortex, a rather end-stage non-specific finding in many cerebellar disorders. However, findings supporting an immune-mediated pathogenesis include diffuse infiltration mainly of T-lymphocytes within the cerebellar white matter as well as marked perivascular cuffing with inflammatory cells [43]. The peripheral nervous system also shows sparse lymphocytic infiltrates with perivascular cuffing being observed in sural nerve biopsy of patients with gluten neuropathy [48–50], in dorsal root ganglia in patients with sensory neuronopathy [55–57] and in patients with gluten myopathy [51]. GRD patients produce an immune response to gluten involving both the innate and adaptive arm of the immune system [71, 75]. Antibodies to gliadin are part of this response, and their systemic levels appear to mirror the immune reaction triggered by gluten in the intestine including their reduction in response to a clinical improvement of the intestinal mucosa. There is cross-reactivity of these antibodies with antigenic epitopes on Purkinje cells. Purkinje cells of both human and rat origin are

recognised from serum of patients with GA and patients with CD but no neurological symptoms [45]. This reactivity can also be seen using polyclonal AGA and the reactivity eliminated by absorption with crude gliadin. When using sera from patients with GA, there is evidence of additional antibodies targeting Purkinje cell epitopes since elimination of AGA alone is not sufficient to eliminate such reactivity. There is evidence that the additional antibodies that may be causing such reactivity are antibodies against one or more transglutaminase isoenzymes (TG2, TG3, TG6) [10].

TG2 belongs to a family of enzymes that covalently cross-link or modify proteins through transamidation, deamidation or esterification of a peptide-bound glutamine residue [5]. Notably, the deamidation reaction may occur in preference over the transamidation reaction, even under conditions that should favour amine incorporation, and this appears to be substrate sequence context-dependent [122]. Gluten proteins (from wheat, barley and rye), the immunological trigger of GRD, are glutamine-rich donor substrates amenable to deamidation. Deamidation of gluten peptides enhances binding with disease-relevant HLAs and thereby enhances presentation, leading to the development of gluten-specific Th1-like $CD4^+$ T cells [71, 128]. The resulting inflammatory cytokine environment drives TG2 expression through direct transcriptional regulation [5, 101], thereby further increasing the production of the immunological trigger. Therefore, activation of TG2 and deamidation of gluten peptides appears to be central to disease development and is now well understood at a molecular level [71]. In genetically predisposed individuals, this is at the centre of a destructive chronic inflammatory reaction manifesting as aphthous stomatitis in the oral cavity or villous atrophy in the upper small intestine at sites where the gluten load through food ingestion is high.

Besides the strong gluten-specific T cell response, one of the hallmarks of GRD is a robust IgA autoantibody response to TG2 or TG2 and further TG isozymes [31, 52, 53, 116]. Assessment of serum anti-TG2 antibodies has become an important tool in CD diagnosis, and new ESPGHAN guidelines enable their use as a surrogate marker of disease [68]. However, events leading to the formation of autoantibodies against TG2 or other TG isozymes are less clear. The recent characterisation of an unusual and overwhelming immature plasma cell response in the small intestine goes some way to explain the strict association of gluten-related disorders with autoantibodies to TGs [28]. Notably, intestinal deposits of IgA antibodies targeting TG2 are present at all stages of CD, including early developing CD [78] as well as late-stage refractory CD [113] where patients may be seronegative. With regard to B cell activation and differentiation, the hapten carrier model proposed by Sollid [121], although not formally demonstrated in vivo, appears to hold true, whereby gluten-specific T cells can provide help to TG-specific B cells. This unusual scenario is enabled by the ability of TGs to form stable complexes with gliadin peptides [123] leading to uptake and ultimately presentation of MHC-gliadin complexes by B cells expressing TG-specific IgD. Recent in vitro studies confirmed that this is indeed possible [28]. Given the relative lack of somatic hypermutation of the antibody repertoire present in adult patients that should have undergone extensive affinity maturation [28, 70], questions remain as to the mechanism by which B

cell maturation takes place, and this could involve an extrafollicular pathway [99]. Plasmablasts re-enter the lamina propria via the circulation and form the IgA-secreting plasma cell niche. It is important to keep in mind that B cells have roles beyond antibody production, most notably as highly effective antigen presenting cells for T cell responses. Therefore, B cells may drive clonal expansion of gluten-specific T cells which in turn may support development of B cells specific for TGs as well as deamidated gluten peptides and thereby create an amplification loop. This potentially puts B cells at the centre stage of GRD pathogenesis.

Questions also remain as to the contribution of these autoantibodies to organ-specific deficits. Anti-TG2 antibodies have been shown to be deposited in the small bowel mucosa of patients with GRD and may contribute to the formation of the lesion. Furthermore, such deposits have been found at extraintestinal sites, such as the muscle and liver [85]. Widespread deposition of IgA antibodies has also been found around brain vessels in GA [48–50]. The deposition was most pronounced in the cerebellum, pons and medulla. This finding suggests that such autoantibodies could play a role in the pathogenesis of the whole spectrum of manifestations seen in GRD and that effector functions of antibodies could contribute to tissue damage. IgM antibodies are present in GRD patients and may activate the complement cascade and promote inflammation.

Variations in the specificity of antibodies produced in individual patients could explain the wide spectrum of manifestations. Whilst TG2 has been shown to be the autoantigen in CD [31], the epidermal transglutaminase TG3 has been shown to be the autoantigen in DH [116]. Antibodies against TG6, a primarily brain-expressed transglutaminase [126], have been shown to be present in patients with GA [52, 53]. Similar to anti-TG2 and anti-deamidated gluten peptide antibodies, the production of these anti-TG3 and anti-TG6 antibodies in DH and GA patients, respectively, is gluten-dependent which substantiates the link to a gluten-specific T cell population [59, 60, 116]. In GA and DH, IgA deposits of TG6 and TG3 respectively seem to accumulate in the periphery of blood vessels at sites where in health the respective proteins are absent. Recent data on DH suggests that the deposits originate from immune complexes forming locally as a consequence of enhanced vascular leaking and that TG3 although potentially present in health may normally be rapidly cleared [134]. Furthermore, TG3 within immune complexes retains enzymatic activity and through cross-linking to fibrinogen and cell surface receptors drives innate immune cell activity [124]. Importantly, the demonstration that circulation-derived anti-TG3 antibodies can induce a dermatitis herpetiformis-like pathology in human skin-grafted SCID mice emphasises the central role antibodies play in disease establishment in different organ systems [134]. By inference, this suggests that adaptive immune cell development likely occurs in the gut and is not driven by trafficking of gut-derived T cells to other organ systems. It is likely that vasculature-centred inflammation is also at the heart of GA. Indeed perivascular cuffing with lymphocytes is a common finding in brain tissue from patients with GA but is also seen in peripheral nerve and muscle in patients with gluten neuropathy or myopathy [55–57]). However, it is unclear at present how immune complexes develop and to what extent a compromised blood-brain barrier is a prerequisite to disease development.

In most sera reactive with more than one TG isoenzyme, distinct antibody populations are responsible for such reactivity rather than this being a result of cross-reactivity with different TG isozymes [52, 53]. The absence of cross-reactivity was recently substantiated in an analysis of clonal antibodies constituting the antibody repertoire in CD [70]. This makes shared epitopes less likely to be the cause for B cell development to other TGs and points to the possibility that TG isozymes other than TG2 can be the primary antigen in GRD. All 3 TG isozymes (TG2, TG3, TG6) for which autoantibodies have been described can form thioester-linked complexes with gluten peptides which are thought to be responsible for the B cell response to TG isozymes [123]. This implicates the shared activity of these enzymes rather than their sequence similarity in stimulation of antibody production and explains the exquisite specificity of the antibody response to TG family members. Whilst antibodies targeting other autoantigens have been reported, the development of such antibodies is much more sporadic amongst the GRD patient population [32].

IgA deposition in brain vessels and the pathological finding of perivascular cuffing with inflammatory cells may indicate that vasculature-centred inflammation may compromise the blood-brain barrier, allowing exposure of the CNS to pathogenic antibodies and therefore be the trigger of nervous system involvement. Indeed, TG2 is expressed by smooth muscle and endothelial cells in non-inflamed brain and is an abundant component of the choroid plexus extracellular matrix [4], and autoantibody binding could initiate an inflammatory response. However, expression of anti-TG2 antibodies in mice by themselves did not precipitate CD-like lesions in the small intestine or overt systemic manifestations akin of GRD [27], and no antibody deposition in brain vessels was reported. This may relate to the fact that it involves a specific subset of anti-TG2 antibodies that was not represented by the analysed clonal antibodies. It could also suggest that development of antibodies targeting antigens other than TG2 may be a critical step in the precipitation of specific extraintestinal manifestations as illustrated by anti-TG3 antibodies in DH [134]. It is also possible that additional factors other than the autoantibodies themselves play a role. These may either affect vascular permeability, blood-brain barrier integrity or antigen availability. With regard to the latter, TG2 and other TGs adopt a number of vastly different conformations dependent on biological context [110], and the recognition of TG2 by antibodies is conformation dependent [70, 120], or binding sites of TG2 may be masked in situ by other interaction partners as recently documented [70]. One might speculate that an unrelated infection or other insult that causes local inflammation may in the presence of circulation-derived autoantibodies bring about pathogenic immune complexes at the blood-brain barrier. This hypothesis is consistent with experimental evidence showing that antibody-mediated neuronal damage in mice harbouring pathogenic antibodies does only occur upon compromise of the blood-brain barrier [82]. Furthermore, brain areas affected in experimental animals, and therefore induced functional deficits, differed dependent on the mechanism underlying the breach of the blood-brain barrier [82]. It appears therefore that regionally specific vascular permeability leads to localised neuronal damage. Similarly, localised exposure to pathogenic antibodies may explain why patients with cerebellar ataxia or stiff person syndrome present with similar dysfunctions affecting preferentially the cerebellum or spinal cord, respectively.

It could be argued that development and deposition of antibodies is an epiphenomenon rather than being pathogenic. One method to demonstrate the pathological effect of an antibody is the passive transfer of the disease through antibody injection into a naïve animal. Whilst for only very few antibody-mediated diseases such experimental evidence exists, IgG fractions of patients with anti-GAD ataxia and stiff-man syndrome have been shown to compromise motor function and impair learning in rodents, an effect possibly ascribed to antibodies against GAD [93]. A common problem in such studies is to be able to demonstrate whether it is these specific antibodies or other autoantibodies in the IgG-fraction of patient sera that cause neuronal damage. Using a mouse model, we have recently shown that serum from GA patients and clonal monovalent anti-TG immunoglobulins derived using phage display cause ataxia when injected intraventricularly in mice [10]. The fact that not only Ig fractions but also monospecific scFv's mediate functional deficits shows that there is no requirement for complement activation or for the engagement of Fc receptors on Fc-receptor-bearing cells in the brain. These data therefore provide evidence that anti-TG immunoglobulins (derived from patients) compromise neuronal function in selected areas of the brain once exposed to the CNS and suggest that this involves an immune system independent mode of action.

A bias of the immune response towards TG6 in GRD patients presenting with neurological deficits [52, 53, 59, 60] implicates neuronal TG6 in pathogenesis, at least of GA but possibly also other neurological problems. Further support for this notion comes from the identification of mutations in the gene encoding TG6 in families with autosomal dominant ataxia [88, 131]. This form of spinocerebellar ataxia is now referred to as SCA35. Clinical features associated with TGM6 mutations are those of late-onset cerebellar ataxia, slow progression of gait and limb ataxia, hyperreflexia and cerebellar degeneration but with no cognitive impairment, autonomic and peripheral nerve involvement or epilepsy [86, 131]. This is in keeping with the presentation in patients with immune-mediated cerebellar ataxia (GA) and provides strong evidence for an essential function of TG6 in the CNS. TG6 is, however, expressed by other cells including various epithelia [125] and one of the TGM6 mutations also associated with acute myeloid leukaemia [103]. Functional data on the physiological role of TG6 protein are sparse at present. We have begun to characterise the enzyme biochemically and analyse the gene expression pattern during development, which identified a complex system with splice variants that are differentially expressed and presumably functionally distinct [125]. Using molecular modelling and biochemical assays, we have shown that TG6 is regulated by GTP and Ca^{2+} similar to TG2 and adopts compact or extended conformations with transamidation activity, respectively [125]. The expression of TG6 during CNS development demonstrated an association with neurogenesis, and this was further confirmed by in vitro differentiation of neuronal precursor cells [125]. All single nucleotide exchanges reported to date lead to alteration of amino acid residues that are strictly conserved in TG6 amongst different species. Based on structural modelling and biochemical analysis [125], we hypothesise that the biological significance of TGM6 mutations lies in the impairment of regulation of transamidation activity. This implicates TG6 in an extracellular function that is critical to neuronal

differentiation and survival. However, how cross-linking or modification of extracellular proteins contributes to neuronal survival remains to be identified. Autoantibody binding may sequester TG6 or block its activity and thereby act as a competitive inhibitor of enzyme function.

Conclusions

GRD include immune-mediated diseases triggered by ingestion of gluten proteins. Whilst coeliac disease has been the most comprehensively studied of all GRD, to fully understand the immunological aftermath from gluten ingestion, there is a need to further study extraintestinal manifestations. In addition, there is a need for the early identification of those patients that are specifically at risk of irreversible complications (e.g. gluten ataxia). To that effect, new diagnostic tools are now becoming available (e.g. antibodies against TG6) which may make a more reliable identification of those patients with neurological manifestations a reality. Up to 40 % of patients presenting to the gastroenterologist who are ultimately diagnosed with CD also have antibodies against TG6 in addition to antibodies against TG2. This subgroup of patients with classic CD presentation may well be the ones susceptible to the development of neurological dysfunction if they continue to consume gluten, although this remains to be shown in longitudinal studies. The presence of gastrointestinal symptoms, however, offers a major potential advantage to this group, as it substantially increases their chances of being diagnosed and treated early, whereas the diagnosis of those patients presenting purely with extraintestinal manifestations may be more difficult.

References

1. Abele M, Bürk K, Schöls L, et al. The aetiology of sporadic adult-onset ataxia. Brain. 2002;125:961–8.
2. Abele M, Schols L, Schwartz S, et al. Prevalence of antigliadin antibodies in ataxia patients. Neurology. 2003;60:1674–5.
3. Addolorato G, Di Giuda D, De Rossi G, et al. Regional cerebral hypoperfusion in patients with celiac disease. Am J Med. 2004;116:312–7.
4. Aeschlimann D, Paulsson M. Cross-linking of laminin-nidogen complexes by tissue transglutaminase. A novel mechanism for basement membrane stabilization. J Biol Chem. 1991; 266(23):15308–17.
5. Aeschlimann D, Thomazy V. Protein crosslinking in assembly and remodelling of extracellular matrices: the role of transglutaminases. Connect Tissue Res. 2000;41(1):1–27.
6. Anheim M, Degos B, Echaniz-Laguna A, et al. Ataxia associated with gluten sensitivity, myth or reality? Rev Neurol. 2006;162:214–21.
7. Beversdorf D, Moses P, Reeves A, et al. A man with weight loss, ataxia, and confusion for 3 months. Lancet. 1996;347:448.
8. Bhatia KP, Brown P, Gregory R, et al. Progressive myoclonic ataxia associated with celiac disease. Brain. 1995;18:1087–93.

9. Binder H, Solitaire G, Spiro H. Neuromuscular disease in patients with steatorrhoea. Gut. 1967;8:605–11.
10. Boscolo S, Lorenzon A, Sblattero D, et al. Anti Transglutaminase antibodies cause ataxia in mice. PLoS One. 2010;5(3), e9698. doi:10.1371/journal.pone.0009698.
11. Brannagan TH, Hays AP, Chin SS, et al. Small-fiber neuropathy/neuronopathy associated with celiac disease: skin biopsy findings. Arch Neurol. 2005;62:1574–8.
12. Briani C, Zara G, Alaedini A, et al. Neurological complications of coeliac disease and autoimmune mechanisms: a prospective study. J Neuroimmunol. 2008;195:171–5.
13. Bundey S. Adult celiac disease and neuropathy. Lancet. 1967;1:851–2.
14. Bürk K, Bösch S, Müller CA, et al. Sporadic cerebellar ataxia associated with gluten sensitivity. Brain. 2001;124:1013–9.
15. Bürk K, Melms A, Schulz JB, et al. Effectiveness of intravenous immunoglobulin therapy in cerebellar ataxia associated with gluten sensitivity. Ann Neurol. 2001;50:827–8.
16. Bushara KO, Goebel SU, Shill H, et al. Gluten sensitivity in sporadic and hereditary ataxia. Ann Neurol. 2001;49:540–3.
17. Catassi C, Gatti S, Fasano A. The new epidemiology of celiac disease. J Paed Gastroenterol Nutr. 2014;59:S7–9.
18. Chapman RWG, Laidlow JM, Colin-Jones D, et al. Increased prevalence of epilepsy in coeliac disease. BMJ. 1978;2:250–1.
19. Chin RL, Sander HW, Brannagan TH, et al. Celiac neuropathy. Neurology. 2003;60:1581–5.
20. Chin RL, Tseng VG, Green PHR, et al. Multifocal axonal polyneuropathy in celiac disease. Neurology. 2006;66:1923–5.
21. Coers C, Telerman-Toppet N, Cremer M. Regressive vacuolar myopathy in steatorrhea. Arch Neurol. 1971;24:217–27.
22. Collin P, Pirttila T, Nurmikko T, et al. Celiac disease, brain atrophy and dementia. Neurology. 1991;41:372–5.
23. Combarros O, Infante J, Lopez-Hoyos M, et al. Celiac disease and idiopathic cerebellar ataxia. Neurology. 2000;54:2346.
24. Cooke WT, Thomas-Smith W. Neurological disorders associated with adult coeliac disease. Brain. 1966;89:683–722.
25. Cronin CC, Jackson LM, Feighery C, et al. Coeliac disease and epilepsy. QJM. 1998;91:303–8.
26. Deconinck N, Scaillon M, Segers V, et al. Opsoclonus-myoclonus associated with celiac disease. Pediatr Neurol. 2006;34:312–4.
27. Di Niro R, Sblattero D, Florian F, et al. Anti-idiotypic response in mice expressing human autoantibodies. Mol Immunol. 2008;45(6):1782–91.
28. Di Niro R, Mesin L, Zheng NY, et al. High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med. 2012;18(3):441–5.
29. Dick DJ, Abraham D, Falkous G, et al. Cerebellar ataxia in coeliac disease- no evidence of a humoral aetiology. Postgrad Med J. 1995;71:186.
30. Dicke WK, Weijers HA, Van De Kamer JH. Coeliac disease II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. Acta Paediatr. 1953;42:34–42.
31. Dietrich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nature Med. 1997;3:797–801.
32. Dieterich W, Esslinger B, Trapp D, et al. Cross linking to tissue transglutaminase and collagen favours gliadin toxicity in coeliac disease. Gut. 2006;55(4):478–84.
33. Elders C. Tropical sprue and pernicious anaemia, aetiology and treatment. Lancet. 1925;1:75–7.
34. Finelli P, McEntee W, Ambler M, et al. Adult celiac disease presenting as cerebellar syndrome. Neurology. 1980;30:245–9.
35. Fois A, Vascotto M, Di Bartolo RM, et al. Celiac disease and epilepsy in pediatric patients. Childs Nerv Syst. 1994;10:450–4.

36. Gabrielli M, Cremonini F, Fiore G, et al. Association between migraine and celiac disease: results from a preliminary case-control and therapeutic study. Am J Gastroenterol. 2003;98: 625–9.
37. Gee S. On the coeliac affection. St Bartholomew's Hosp Rep. 1888;24:17–20.
38. Gibbons CH, Freeman R. Autonomic neuropathy and celiac disease. J Neurol Neurosurg Psychiatry. 2005;76:579–81.
39. Gobbi G, Bouquet F, Greco L, et al. Coeliac disease, epilepsy and cerebral calcifications. Lancet. 1992;340:439–43.
40. Gobbi G. Coeliac disease, epilepsy and cerebral calcifications. Brain Dev. 2005;27:189–200.
41. Hadjivassiliou M, Gibson A, Davies-Jones GAB, et al. Is cryptic gluten sensitivity an important cause of neurological illness? Lancet. 1996;347:369–71.
42. Hadjivassiliou M, Chattopadhyay AK, Davies-Jones GAB, et al. Neuromuscular disorder as a presenting feature of celiac disease. J Neurol Neurosurg Psychiatry. 1997;63:770–5.
43. Hadjivassiliou M, Grunewald RA, Chattopadhyay AK, et al. Clinical, radiological, neurophysiological and neuropathological characteristics of gluten ataxia. Lancet. 1998;352: 1582–5.
44. Hadjivassiliou M, Grünewald RAG, Lawden M, et al. Headache and CNS white matter abnormalities associated with gluten sensitivity. Neurology 2001;56:385–8.
45. Hadjivassiliou M, Boscolo S, Davies-Jones GAB, et al. The humoral response in the pathogenesis of gluten ataxia. Neurology. 2002;58:1221–6.
46. Hadjivassiliou M, Grünewald RA, Sharrack B, et al. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. Brain. 2003;126:685–91.
47. Hadjivassiliou M, Davies-Jones GAB, Sanders DS, et al. Dietary treatment of gluten ataxia. J Neurol Neurosurg Psychiatry. 2003;74(9):1221–4.
48. Hadjivassiliou M, Maki M, Sanders DS, et al. Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. Neurology. 2006;66:373–7.
49. Hadjivassiliou M, Grunewald RA, Kandler RH, et al. Neuropathy associated with gluten sensitivity. J Neurol Neurosurg Psychiatry. 2006;77:1262–6.
50. Hadjivassiliou M, Kandler RH, Chattopadhyay AK, et al. Dietary treatment of gluten neuropathy. Muscle Nerve. 2006;34:762–6.
51. Hadjivassiliou M, Chattopadhyay AK, Grünewald RA, et al. Myopathy associated with gluten sensitivity. Muscle Nerve. 2007;35:443–50.
52. Hadjivassiliou M, Aeschlimann P, Strigun A, et al. Autoantibodies in gluten ataxia recognise a novel neuronal transglutaminase. Ann Neurol. 2008;64:332–43.
53. Hadjivassiliou M, Sanders DS, Woodroofe N, et al. Gluten ataxia. Cerebellum. 2008;7: 494–8.
54. Hadjivassiliou M, Aeschlimann P, Sanders DS, et al. Antibodies against TG6 as the only serological marker of gluten ataxia. Proceedings of the 13th international coeliac disease symposium. Amsterdam; 2009. April 09.1:75.
55. Hadjivassiliou M, Sanders DS, Grunewald RA, et al. Gluten sensitivity: from gut to brain. Lancet Neurol. 2010;9:318–30.
56. Hadjivassiliou M, Rao DS, Wharton SB, et al. Sensory ganglionopathy due to gluten sensitivity. Neurology. 2010;75:1003–8.
57. Hadjivassiliou M, Aeschlimann D, Grunewald RA, et al. GAD antibody associated neurological illness and its relationship to gluten sensitivity. Acta Neurol Scand. 2010. doi:10.1111/J.1600-0404.2010.01356.x.
58. Hadjivassiliou M, Sanders DS, Hoggard N. Magnetic resonance imaging and spectroscopy of the cerebellum in patients with celiac disease and minor neurological complains. Proceedings of the 14th international coeliac disease symposium 2011, Oslo, June 2011. p. 30.
59. Hadjivassiliou M, Hoggard N, Currie S, Aeschlimann P, Sanders DS, Aeschlimann DP. Neurological dysfunction in patients with newly diagnosed coeliac disease: a large prospective study. Proceedings of the 15th international celiac disease symposium, Chicago; 2013. p. A124.

60. Hadjivassiliou M, Aeschlimann P, Sanders DS, Maki M, Kaukinen K, Grunewlad RA, Bandmann O, Woodroofe N, Haddock G, Aeschlimann DP. Transglutaminase 6 antibodies in the diagnosis of gluten ataxia. Neurology. 2013;80:1–6.
61. Hahn JS, Sum JM, Bass D, et al. Celiac disease presenting as gait disturbance and ataxia in infancy. J Child Neurol. 1998;13:351–3.
62. Harper E, Moses H, Lagrange A. Occult celiac disease presenting as epilepsy and MRI changes that responded to gluten-free diet. Neurology. 2007;68:533.
63. Henriksson KG, Hallert C, Norrby K, et al. Polymyositis and adult celiac disease. Acta Neurol Scand. 1982;65:301–19.
64. Hermaszewski RA, Rigby S, Dalgleish AG. Coeliac disease presenting with cerebellar degeneration. Postgrad Med J. 1991;67:1023–4.
65. Holmes GKT. Neurological and psychiatric complications in coeliac disease. In: Gobbi G, Anderman F, Naccarato S, Banchini G, editors. Epilepsy and other neurological disorders in coeliac disease. London: John Libbey; 1997.
66. Hu WT, Murray JA, Greenway MC, et al. Cognitive impairment and celiac disease. Arch Neurol. 2006;63:1440–6.
67. Hunt KA. Newly identified genetic risk variants for coeliac disease related immune response. Nat Genet. 2008;40:395–402.
68. Husby S, Koletzko S, Korponay-Szabó IR, et al. ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136–60.
69. Ihara M, Makino F, Sawada H, et al. Gluten sensitivity in Japanese patients with adult-onset cerebellar ataxia. Intern Med. 2006;45:135–40.
70. Iversen R, Di Niro R, Stamnaes J, Lundin KE, Wilson PC, Sollid LM. Transglutaminase 2-specific autoantibodies in celiac disease target clustered, N-terminal epitopes not displayed on the surface of cells. J Immunol. 2013;190(12):5981–91.
71. Jabri B, Sollid LM. Tissue-mediated control of immunopathology in coeliac disease. Nat Rev Immunol. 2009;9(12):858–70.
72. Jacob S, Zarei M, Kenton A, et al. Gluten sensitivity and neuromyelitis optica: two case reports. J Neurol Neurosurg Psychiatry. 2005;76:1028–30.
73. Jarius S, Jacob S, Waters P, et al. Neuromyelitis optica in patients with Gluten sensitivity associated with antibodies to aquaporin-4. J Neurol Neurosurg Psychiatry. 2008;79:1084.
74. Johnson AM, Dale RC, Wienholt L, Hadjivassiliou M, Aeschlimann D, Lawson JA. Coelaic disease, epilepsy, and cerebral calcifications: association with TG6 autoantibodies. Develop Med Child Neurol. 2012. doi:10.1111/j.469-8749.2012.04369.x.
75. Junker Y, Zeissig S, Kim SJ, et al. Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. J Exp Med. 2012;209(13):2395–408.
76. Kaplan JG, Pack D, Horoupian D. Distal axonopathy associated with chronic gluten enteropathy: a treatable disorder. Neurology. 1988;38:642–5.
77. Kelkar P, Ross M, Murray J. Mononeuropathy multiplex associated with celiac disease. Muscle Nerve. 1996;19:234–6.
78. Kaukinen K, Peräaho M, Collin P, et al. Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study. Scand J Gastroenterol. 2005;40(5):564–72.
79. Kepes JJ, Chou SM, Price LW. Progressive multifocal leukoencephalopathy with 10-year survival in a patient with nontropical sprue. Neurology. 1975;25:1006–12.
80. Kieslich M, Errazuriz G, Rosselt HG, et al. Brain white matter lesions in Celiac disease: a prospective study in diet treated patients. Paediatrics. 2001;108:E21.
81. Kinney HC, Burger PC, Hurwitz BJ, et al. Degeneration of the central Nervous system associated with celiac disease. J Neurol Sci. 1982;53:9–22.
82. Kowal C, Degiorgio LA, Lee JY, et al. Human lupus autoantibodies against NMDA receptors mediate cognitive impairment. Proc Natl Acad Sci U S A. 2006;103(52):19854–9.

83. Kristoferitsch W, Pointer H. Progressive cerebellar syndrome in adult coeliac disease. J Neurol. 1987;234:116–8.
84. Korponay-Szabo IR, Laurila K, Szondy Z, et al. Missing endomysial and reticulin binding of celiac antibodies in transglutaminase 2 knockout tissues. Gut. 2003;52:199–204.
85. Korponay-Szabó IR, Halttunen T, Szalai Z, et al. *In vivo* targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut. 2004;53:641–8.
86. Li M, Pang SYY, Song Y, Kung MHW, Ho SL, Sham PC. Whole exome sequencing identifies a novel mutationin the transglutaminase 6 gene for spinocerebellar ataxia in a Chinese family. Clin Genet. 2013;83:269–73.
87. Lu CS, Thompson PD, Quin NP, et al. Ramsay Hunt syndrome and coeliac disease: a new association. Mov Disord. 1986;1:209–19.
88. Ludvigsson JF, Olsson T, Ekbom A, et al. A population based study of celiac disease, neurodegenerative and neuroinflammatory diseases. Alliment Pharmacol Ther. 2007;25:1317–27.
89. Ludvigsson JF, Zingone F, Tomson T, Ekbom A, Ciacci C. Increased risk of epilepsy in biopsy-verified celiac disease. Neurology. 2012;78:1401–7.
90. Lundin KE, Sollid LM. Advances in coeliac disease. Curr Opin Gastroenterol. 2014;30(2):154–62.
91. Luostarinen LK, Collin PO, Paraaho MJ, et al. Coeliac disease in patients with cerebellar ataxia of unknown origin. Ann Med. 2001;33:445–9.
92. Luostarinen L, Himanen SL, Luostarinen M, et al. Neuromuscular and sensory disturbances in patients with well treated celiac disease. J Neurol Neurosurg Psychiatry. 2003;74:490–4.
93. Manto MU, Laute MA, Aguera M, et al. Effects of anti-glutamic acid decarboxylase antibodies associated with neurological diseases. Ann Neurol. 2007;61:544–51.
94. Marsh M. Gluten, major histocompatibility complex and the small intestine. Gastroenterology. 1992;102:330–54.
95. Marks J, Shuster S, Watson AJ. Small bowel changes in dermatitis herpetiformis. Lancet. 1966;ii:1280–2.
96. Mata S, Renzi D, Pinto F, et al. Anti-tissue transglutaminase IgA antibodies in peripheral neuropathy and motor neuronopathy. Acta Neurol Scand. 2006;114:54–8.
97. Mavroudi A, Karatza E, Papastavrou T, et al. Successful treatment of Epilepsy and celiac disease with a gluten-free diet. Pediatr Neurol. 2005;33:292–5.
98. Morris JS, Ajdukiewicz AB, Read AE. Neurological disorders and adult celiac disease. Gut. 1970;11:549–54.
99. Mesin L, Sollid LM, Di Niro R. The intestinal B-cell response in celiac disease. Front Immunol. 2012;3:313.
100. Muller AF, Donnelly MT, Smith CML, et al. Neurological complications of Coeliac disease-a rare but continuing problem. Am J Gastroenterol. 1996;91:1430–5.
101. Nurminskaya MV, Belkin AM. Cellular functions of tissue transglutaminase. Int Rev Cell Mol Biol. 2012;294:1–97.
102. Paltola M, Kaukinen K, Dastidar P, et al. Hippocampal sclerosis in refractory temporal lobe epilepsy is associated with gluten sensitivity. J Neurol Neurosurg Psychiatry. 2009;80(6):626–30. doi:10.1136/jnnp.2008.148221.
103. Pan LL, Huang YM, Wang M, et al. Positional cloning and next-generation sequencing identified a TGM6 mutation in a large Chinese pedigree with acute myeloid leukaemia. Eur J Human Genet. 2015;23(2):218–23. doi:10.1038/ejhg.2014.67.
104. Paulley JW. Observations on the aetiology of idiopathic steatorrhoea, jejunal and lymph node biopsies. Br Med J. 1954;2:1318–21.
105. Reed AC, Ash JE. Atypical sprue. Arch Intern Med. 1927;40:786–99.
106. Pellecchia MT, Scala R, Filla A, et al. Idiopathic cerebellar ataxia associated with celiac disease: lack of distinctive neurological features. J Neurol Neurosurg Psychiatry. 1999;66:32–5.
107. Pellecchia MT, Scala R, Perretti A, et al. Cerebellar ataxia associated with subclinical celiac disease responding to gluten-free diet. Neurology. 1999;53:1606–7.

108. Pennisi G, Lanza G, Giuffrida S, et al. Excitability of the motor cortex in de novo patients with celiac disease. PLoS One. 2014. doi:10.1371/journal.pone.0102790.
109. Pereira AC, Edwards MJ, Buttery PC, et al. Choreic syndrome and coeliac disease: a hitherto unrecognised association. Mov Disord. 2004;19(4):478–82.
110. Pinkas DM, Strop P, Brunger AT, Khosla C. Transglutaminase 2 undergoes a large conformational change upon activation. PLoS Biol. 2007;5(12), e327.
111. Ranua J, Luoma K, Auvinen A, et al. Celiac disease-related antibodies in an epilepsy cohort and matched reference population. Epilepsy Behav. 2005;6:388–92.
112. Romanos J, Rosén A, Kumar V, et al. PreventCD Group. Improving coeliac disease risk prediction by testing non-HLA variants additional to HLA variants. Gut. 2014;63(3):415–22.
113. Salmi TT, Collin P, Korponay-Szabó IR, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut. 2006;55(12):1746–53.
114. Sander HW, Magda P, Chin RL, et al. Cerebellar ataxia and celiac disease. Lancet. 2003; 362:1548.
115. Sanders DS, Patel D, Stephenson TJ, et al. A primary care cross-sectional study of undiagnosed adult celiac disease. Eur J Gastro Hep. 2003;15:407–13.
116. Sárdy M, Kárpáti S, Merkl B, Paulsson M. Epidermal transglutaminase (TGase3) is the autoantigen of Dermatitis Herpetiformis. J Exp Med. 2002;195:747–57.
117. Sarrigiannis PG, Hoggard N, Aeschlimann D, Sandres DS, Grunewlad RA, Unwin ZC, Hadjivassiliou M. Myoclonus ataxia and refractory coeliac disease. Cerebellum Ataxias. 2014. www.cerebellumandataxias.com/content/1/1/11.
118. Selva-O'Callaghan A, Casellas F, De Torres I, et al. Celiac disease and antibodies associated with celiac disease in patients with inflammatory myopathy. Muscle Nerve. 2007;35:49.
119. Serratrice J, Disdier P, De Roux C, et al. Migraine and celiac disease. Headache. 1998;38: 627–8.
120. Simon-Vecsei Z, Király R, Bagossi P, et al. A single conformational transglutaminase 2 epitope contributed by three domains is critical for celiac antibody binding and effects. Proc Natl Acad Sci U S A. 2012;109(2):431–6.
121. Sollid LM, Molberg O, McAdam S, Lundin KE. Autoantibodies in coeliac disease: tissue transglutaminase – guilt by association? Gut. 1997;41:851–2.
122. Stamnaes J, Fleckenstein B, Sollid LM. The propensity for deamidation and transamidation of peptides by transglutaminase 2 is dependent on substrate affinity and reaction conditions. Biochim Biophys Acta. 2008;1784(11):1804–11.
123. Stamnaes J, Dorum S, Fleckenstein B, Aeschlimann D, Sollid LM. Gluten T cell epitope targeting by TG3 and TG6; implications for dermatitis herpetiformis and gluten ataxia. Amino Acids. 2010;39(5):1183–91.
124. Taylor TB, Schmidt LA, Meyer LJ, Zone JJ. Transglutaminase 3 present in the IgA aggregates in dermatitis herpetiformis skin is enzymatically active and binds soluble fibrinogen. J Invest Dermatol. 2015;135(2):623–5.
125. Thomas H, Beck K, Adamczyk M, et al. Transglutaminase 6: a protein associated with central nervous system development and motor function. Amino Acids. 2013;44(1):161–77.
126. Tison F, Arne P, Henry P. Myoclonus and adult celiac disease. J Neurol. 1989;236:307–8.
127. Toti P, Balestri P, Cano M, et al. Celiac disease with cerebral calcium and silica deposits: X-ray spectroscopic findings, an autopsy study. Neurology. 1996;46:1088–92.
128. Tye-Din JA, Stewart JA, Dromey JA, et al. Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. Sci Transl Med. 2010;2(41):41ra51.
129. Ventura A, Magazu G, Gerarduzzi T, Greco L. Coeliac disease and the risk of autoimmune disorders. Gut. 2002;51:897–8.
130. Volta U, Bardella MT, Calabro A, Troncone R, Corazza GR. An Italian prospective multicentre survey on patients suspected of having non-celiac gluten sensitivity. BMC Med. 2014;12:85.
131. Wang JL, Yang X, Xia K, et al. TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing. Brain. 2010;133:3510–8.

132. Ward ME, Murphy JT, Greenberg GR. Celiac disease and spinocerebellar degeneration with normal vitamin E status. Neurology. 1985;35:1199–201.
133. Woltman HW, Heck FJ. Funicular degeneration of the spinal cord without pernicious anemia. Arch Intern Med. 1937;60:272–300.
134. Zone JJ, Schmidt LA, Taylor TB, et al. Dermatitis herpetiformis sera or goat anti-transglutaminase-3 transferred to human skin-grafted mice mimics dermatitis herpetiformis immunopathology. J Immunol. 2011;186(7):4474–80.

Chapter 16
The Neurology of Autoimmune Pernicious Anaemia (Subacute Combined Degeneration)

Laura Edwards

Abstract The link between pernicious anaemia and subacute combined degeneration of the spinal cord has been recognised for nearly a century. The most common neurological presenting symptoms and signs of vitamin B12 deficiency are distal paraesthesia and loss of vibration and joint position sense. A multitude of other neuropsychiatric manifestations have been described, often responding well to treatment with vitamin B12.

This chapter discusses the history of pernicious anaemia and subacute combined degeneration of the cord, the common and less common neuropsychiatric manifestations and the historically and currently recommended treatment regimens. It also touches briefly on some of the current controversies and unknowns, including the apparent dissociation between the haematologic and neurologic manifestations, the role of vitamin B12 deficiency in cognitive impairment/dementia, the use of autoantibody (anti-intrinsic factor and parietal cell antibodies) detection in the diagnosis of pernicious anaemia and the utilisation of different assays to identify B12 deficiency.

Keywords Vitamin B12 deficiency • Pernicious anaemia • Subacute combined degeneration of the spinal cord • Peripheral neuropathy • Cognitive impairment

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Disclaimer In many of the papers reviewed, it has been difficult to differentiate between B12 deficiency due to pernicious anaemia (PA) and that due to other causes. Where possible, I have clarified which is being discussed.

As it is still argued that the majority of cases of B12 deficiency in adulthood are due to PA, I felt it was rash to exclude those papers where a diagnosis of PA either could not be definitively made or was not explicitly stated.

Furthermore while there are many papers written about B12 deficiency and, occasionally, PA in childhood, this chapter is focussing on the manifestations in adults.

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Abbreviations

PA	Pernicious anaemia
SACD	Subacute combined degeneration of the cord

Introduction

Pernicious anaemia (PA) is an autoimmune disease of the gastric mucosa, where autoantibodies are produced against intrinsic factor and parietal cells, causing a failure of secretion of gastric acid and of intrinsic factor. Both contribute to the malabsorption of vitamin B12, which is liberated from food by gastric acid, bound to intrinsic factor and then absorbed in the terminal ileum. It is important to note that a very small amount of B12 can also be absorbed independent of intrinsic factor [1], which in fact is important in some of the treatment effects of high-B12 diets in the treatment of PA before the introduction of parenteral B12 therapy.

Lack of vitamin B12 has several consequences. B12 is a coenzyme in two key reactions, converting homocysteine to methionine and converting methylmalonyl coenzyme A to succinyl coenzyme A. The first pathway contributes to DNA and RNA synthesis; therefore, its disruption affects rapidly dividing cells first (e.g. blood cells, causing an anaemia and pancytopenia; cells of the gastrointestinal tract mucosa, contributing to diarrhoea and malabsorption). Lack of B12 also causes build-up of the upstream reagents in the pathways – homocysteine and methylmalonic acid; measuring these substances can be helpful in identifying cases of B12 deficiency (see later).

B12 itself is a water-soluble vitamin also known as cobalamin. It has a central cobalt atom in a porphyrin ring. Of note, the cobalt is irreversibly oxidised by nitrous oxide, which is why nitrous oxide anaesthesia can often unmask hitherto unsuspected B12 deficiency [2–6].

All B12-rich foods in the human diet are of animal origin – from meat, fish or dairy products. The daily requirement for vitamin B12 is low (around 1.5 micrograms per day [7], which is provided many times over by the average Western diet day [8]. The body stores in most people are high (3–5 mg [9]) and the biological half-life is approximately one year, with the average person losing approximately 0.13 % of body stores per day [10, 11]. This means that it requires exceptional dietary restriction or failure of absorption to develop B12 deficiency, and the signs and symptoms can take several years to develop after the inciting insult.

The exact mechanisms by which B12 deficiency disrupts nervous system function are unknown. It is generally accepted to be related to a defect in myelin synthesis, possibly through impaired DNA synthesis, the incorporation of abnormal fatty acids into myelin, the presence of toxin(s) or lack of some protective mechanism(s) [12, 13]. Accumulated homocysteine is also thought to be neurotoxic and vasculotoxic [14]. The overall result is initial demyelination of nerves, leading to axonal degeneration if untreated.

Historical Aspects of Pernicious Anaemia and Subacute Combined Degeneration of the Spinal Cord

Anaemia and Gastric Atrophy

In the 1800s, anaemia became a source of much interest and excitement ("a very peculiar disease, which has excited little attention among medical men, and which has altogether been overlooked by any English author with whose writing I am acquainted" [15]); and Thomas Addison, in 1849, presented his findings of a series of cases which have since been suggested to combine features of Addison's disease (adrenal pathology) and possibly also (pernicious) anaemia – "relaxation and flabbiness, rather than wasting of the flesh" and "a manifest paleness of the countenance" [16].

The anaemia was investigated further by other authors, without the adrenal findings and with a variety of suggestions as to the underlying pathology – describing anaemic patients with diarrhoea and a "pale yellowish" complexion, struggling with fatigue and lower limb sensory symptoms. Flint, in 1860, hypothesised that "in these cases there exists degenerative diseases of the glandular tubuli of the stomach" [17], and his theory was borne out by post-mortem findings showing marked atrophy of the stomach which "prevented digestion of the albuminous materials of the food" [18]. A further case series described features arguably recognisable as PA – leukopenia ("blood film with diminution in the number of the white globules"), neurological symptoms ("dropsy appeared in the legs"), gastrointestinal symptoms ("occasional diarrhoea or tendency to it"), time course ("slow progressive anaemia"), demographics ("occurring in persons of middle age") and the, at this point in history, invariably fatal course of the disease [19].

Early SACD

Over the next few decades, there were several publications describing the key findings of SACD. Putnam and Dana, both USA-based, published independently strikingly similar accounts of the disease [20, 21], which Stewart then brought to notice in the UK [22]; Dana then proceeded to describe a series of cases in more detail [23], as did Russell, the latter using cases seen at the National Hospital in London [24]. These descriptions give the key findings of SACD as recognised today.

At that time, the disease ran its course, from presentation to death, within approximately two years [20, 21]. It was recognised to occur more in females than in males and typically in persons of around middle age [21, 23], was commonly associated with diarrhoea and anaemia – even pernicious/macrocytic anaemia, although the authors recognised a discrepancy between the severity of the haematologic and neurologic manifestations [21–24]. Dana, in fact, stated that "the existence of marked anaemia, and especially of pernicious anaemia is, taken with ataxia, paralysis and rapid course, pathognomonic" [23].

The presenting neurologic symptoms were most commonly paraesthesia or "numbness of the extremities", most markedly in the lower limbs, sometimes associated with weakness and ataxia, with the symptoms worsening and moving more proximally over time. The upper limbs tended to be less involved and to become symptomatic later than the lower limbs. Ataxia and impairment of joint position sense could be very severe, often contributing more to loss of mobility at an early stage than did weakness. Patients often developed marked spasticity in the lower limbs, with upgoing plantars, becoming paraplegic in the "intermediate" stage. Later, flaccid quadriplegia with loss of bowel and bladder control developed. These later stages were sometimes associated with "mental symptoms approaching dementia" and occasionally other cerebral signs and symptoms such as psychosis and cranial nerve palsies, but in the main, cerebral manifestations were rare [20, 21, 23–25].

The pathological findings from these cases showed essentially normal brain tissue but marked changes in the spinal cord and, to a lesser extent, the peripheral nerves, summarised by Putnam as showing:

1. "A relatively chronic sclerosis in the posterior and lateral centripetal and centrifugal long tracts
2. A more acute and recent degenerative change in adjoining areas, partly diffuse and partly systemic in origin
3. A diffuse degeneration of varying severity and uncertain duration in the ganglionic matter of the cord, and probably the intervertebral ganglia
4. Degeneration of a moderate degree in the nerve roots and peripheral nerves" [21]

These findings were consistent with those from other authors of the period, who further highlighted:

- The particular involvement of the mid-dorsal region of the cord
- Lesions reaching up as high as the medulla but lessening as one travelled caudally
- The essentially non-inflammatory nature of the lesions
- The presence of demyelination
- The primary involvement of posterior columns, cerebellar and pyramidal tracts
- Secondary degeneration in the "long tracts" of the cord in more long-standing cases which had a more "homogeneous distribution"
- Posterior column involvement antedating anterolateral column involvement by several months [23–26]

Early SACD and PA

Although earlier studies mentioned associations between PA and SACD [23, 24], Hurst, in 1922 and 1924, brought together the twin pathologies more definitively, saying that they "depend upon the same pathological process" and that "Anaemia

with which subacute combined degeneration of the cord is almost always associated is the so-called pernicious form, first described by Addison" and pointing the finger at "oral sepsis, absence of free hydrochloric acid from the stomach contents throughout digestion, and consequent intestinal infection and intoxication".

Hurst emphasised that "no definite line can be drawn between Addison's anaemia accompanied by changes in the spinal cord, and subacute degeneration of the spinal cord accompanied by anaemia. The difference is simply one of degree and depends upon whether a haemolysin or a neurotoxin is more active". He, and others, emphasised the vital importance of looking for gastric acid secretions when performing a workup for SACD, which allowed the exclusion of a variety of differential diagnoses [27, 28], as "achylia gastrica is at least as constant in subacute combined degeneration of the cord as in Addison's anaemia" [26].

By the 1950s, the underlying cause of PA was described as "malabsorption of vitamin B12 due to lack of intrinsic factor consequent on gastric atrophy", and the primary cause of SACD was suggested to be vitamin B12 deficiency, since "administration of vitamin B12 invariably halts the progression of the spinal cord disease, and indeed may reverse some of the neurological signs, particularly if they are of short duration…" while recognising that less than 10 % of PA patients were affected by SACD [29].

Early Identification of Cerebral Manifestations

From the late 1800s through the first third of the 1900s, a variety of manifestations of PA thought to be due to cerebral involvement were described, ranging from mild memory impairment to toxic psychosis but with essentially normal brain findings at post-mortem, at least macroscopically, albeit with "occasionally some wasting of the cortex, with slight thickening of the membranes and compensatory oedema" [20, 24, 25].

In the 1940s and 1950s, white matter lesions, demyelination and "diffuse degeneration" were described in PA patients in the cerebral hemispheres, cerebellar peduncles and optic tracts which appeared to be very similar to those changes seen in the spinal cord [30, 31]. The findings of demyelination and neuronal damage ("acute, severe, chronic, ischaemic, oedematous and fatty changes in varying degree") were independent of the presence or absence of cerebral signs or symptoms [32].

Cerebral manifestations became more widely recognised, with one author saying it was a "well-known fact" that SACD patients "often exhibit mental symptoms that vary from slight irritability and suspiciousness to a marked confusional psychosis" [30]. Other patients had diagnoses of optic atrophy, seizures and delusions related to B12 deficiency [31].

Early Treatments: Previtamin B12

In the late 1800s, good general nutrition was recommended for the treatment of anaemia, including PA [17, 21]. A case report was published where a patient taking ox and calf bone marrow experienced such a resolution in his PA symptoms that he was transformed from a bedridden invalid into a haemodynamically replete, energetic individual who even "enjoyed assisting in ward work such as carrying coals up a long flight of stairs" [33].

Hurst's conviction that PA was due to a combination of achlorhydria and oral sepsis led him to suggest the following (not terribly tempting) regimen for his patients:

1. Remove all teeth and tonsils if they show any trace of infection.
2. Isolate *Streptococcus* from teeth/duodenal contents/faeces/urine; use this to produce a vaccine for the patient.
3. Large volumes of dilute hydrochloric acid and soured milk.
4. Powdered charcoal (to absorb the haemolysin/neurotoxin).
5. Arsenic for symptomatic relief.
6. Blood transfusion.
7. Splenectomy to remove the site of blood destruction.

He claimed good results in both the neurologic and haematologic status of his patients using these treatments but admitted that "the degree of improvement which may occur depends therefore upon the amount of actual destruction of nervous tissue which is already present" [27, 28].

Minot and colleagues were awarded the Nobel Prize for their work which included the introduction of a diet containing "an abundance of liver and muscle meat" for PA patients, resulting in significant haematologic improvement [34]. Liver, of course, has high levels of iron and also provides B12 (a tiny proportion of which can be absorbed by passive diffusion without intrinsic factor [1]) and folic acid [35] so could be seen as a "universal" treatment for the common nutritional anaemias. However, unequivocal neurological improvement remained to be seen "except in the opinions of a few", as somewhat scathingly reported in 1929 [25].

By 1933, it was held that "the anti-anaemic principle contained in liver" (at roughly half a pound per day or, more palatably, a preparation of liver extract [36] or even of marmite [37]) made PA an essentially treatable condition [36]. However, sensitivity to liver extract remained a problem, which was only overcome by the introduction of pure B12, which was identified, characterised and synthesised between the 1940s and 1960s, leading to a revolution in the treatment of PA (reviewed in [38]).

Current Clinical Picture

The clinical manifestations of vitamin B12 deficiency and/or PA were all described over a century ago, and current case reports tend to do little more than reiterate many of these findings. Fortunately, now the condition is more easily recognised

and treated, the majority of patients avoid signs and symptoms which are as extreme and disabling as they would have been in the 1800s and first half of the last century.

The overall prevalence of neuropsychiatric involvement in cases of PA/B12 deficiency varies from study to study – in the studies mentioned below, the frequency of nervous system involvement decreased from 80 % in 1918 [39] to 44 % in 1988 [40], but neurological abnormalities found on examination were reported in 80 % of patients in a large study [41] and neurological symptoms were reported in 98 % of PA answering an online survey in 2014 (although this included some non-specific symptoms such as poor sleep and poor concentration) [42]. The "classical" findings of SACD were estimated to occur in 10 % of PA cases in 1958 and in 16 % in 1980 [29, 43].

A study showed that the median duration of symptoms before presentation was only 4 months but that the majority, 99 cases, were classified at being "mild" neurological impairment, 39 being considered "moderate" and only 15 being classified as "severe" [41].

Table 16.1 details the "top five" signs and symptoms taken from papers with large case series; rarer manifestations are discussed below. This spans almost a century, and it can be seen that, while proportions of patients affected by different signs and symptoms may vary, the most common signs and symptoms remain throughout paraesthesia and impaired joint position and vibration sense. As mentioned in the introduction, it is often difficult to tease out which patients in which study are B12 deficient due to PA and which are deficient due to other causes. Where it can be calculated and identified, this is mentioned in the table below. Only one paper clearly differentiates the symptoms and signs in PA-related B12 deficiency patients and non-PA-related B12 deficiency, which showed that neuropsychiatric abnormalities were more frequent in PA patients (affecting 81 % of PA-B12 deficiency but only 45 % of non-PA-B12 deficiency); the individual signs and symptoms affect fairly low numbers so it is difficult to compare [43].

Paraesthesiae are arguably the most common symptoms at presentation, affecting between 20 and 80 % of patients depending on the study, with the majority being around the 70 % mark [39–42, 44]. This is commonly accompanied by other symptoms, frequently weakness, fatigue, difficulty with gait and clumsiness, and less frequently bowel and bladder symptoms [39–43, 45].

The most common signs are sensory abnormalities and particularly impairment of joint position sense and vibration sense, seen in 10–92 % of cases, again more commonly towards the upper end of this range and occurring much more in the lower than upper limbs [39–41, 43, 45]. There are also frequently abnormalities found in the reflexes, particularly in the lower limbs – commonly diminished or absent tendon reflexes with upgoing plantars, but increased reflexes have also been reported [39, 40, 43, 45]. The clinical findings not uncommonly indicate a combination of peripheral neuropathy and spinal cord pathology, most commonly involving the posterior columns (52 %) but also the anterior and lateral columns [39, 43].

An important finding, discussed in several of the early as well as more recent studies, is that the severity of the anaemia and of the neurological abnormalities is strikingly dissociated. 44 % of patients with diagnosed B12 deficiency but no

Table 16.1 "Top five" neurological signs and symptoms in B12-deficient patients

Study and year	No. and type of patients	Top symptoms	%age patients affected	Top signs	% age of patients affected
Woltmann (1918)	150 PA cases	Paraesthesiae	80	Impaired vibration sense	82
		Urinary incontinence	8.8	Impaired joint position sense	60
		Urinary retention	4.8	Impaired coordination legs	55.2
		Girdle pain	2.8	Rombergism	52
		Taste disturbance	2	Increased patellar reflex	39.2
Shorvon (1980)	50 B12-deficient patients (64 % PA)	Leg paraesthesia	18	Impaired vibration sense, legs	40
		Leg ataxia	8	Extensor plantars	16
		Leg weakness	6	Distal weakness	10
		Leg and arm paraesthesia	6	Impaired joint position sense	10
				Leg ataxia	8
Lindenbaum (1988)	40 non-anaemic B12 deficient patients (68 % proven PA; 15 % probable)	Paraesthesia	75	Impaired vibration sense	77.5
		Weakness	55	Impaired joint position sense	55
		Fatigue	22.5	Impaired touch/pain sense	42.5
		Urinary or faecal incontinence	15	Ataxia	42.5
		Urinary urgency/nocturia	10	Abnormal gait	37.5
Stabler (1990)	145 B12-deficient patients	Paraesthesia	19.3	Impaired vibration sense	14.5
		Ataxia	14.5	Abnormal gait	12.4
		Weakness	4.8	Impaired touch/pain sense	9.7
		Faecal incontinence	1.4	Impaired position sense	7.6
		Impotence	0.7	Hyporeflexia	4.8

Healton (1991)	369 B12-deficient patients (66% proven PA; 12% probable)	Paraesthesia/numbness	13.1	Impaired vibration + position sense	11.1
		Gait ataxia	3.6	Impaired vibration sense	8.2
		Impaired dexterity	1.3	Impaired vibration, cutaneous and position sense	6.2
		Leg weakness	0.5	Ataxia	5.9
		Orthostatic lightheadedness	0.5	Paraparesis	2.1
Hooper (2014)	889 PA patients from online survey	Fatigue	96		
		Clumsiness	66		
		Paraesthesia	66		
		Dizziness	59		
		Headaches	52		

anaemia or macrocytosis had significant neurological symptoms in one study [40]; in another, 58 % of B12-deficient patients were not anaemic but had neuropsychiatric abnormalities [45], and in Healton et al.'s large study, there was actually an inverse correlation between the degree of anaemia and the extent of neurologic impairment, with anaemia being more severe in those without neurologic involvement and the severity of neurologic impairment actually correlating with the haematocrit in non-anaemic patients; non-anaemic patients were also less likely to return to neurologic baseline following B12 therapy [41].

There have been many, many case reports and small case series emphasising the less common presentations of B12 deficiency – often reiterating findings from the earlier papers – including descriptions of:

- Cranial nerve abnormalities [24, 39, 46, 47]
- Movement disorders (choreiform [17, 39, 48, 49], Parkinsonian [3, 50], myoclonic [17, 39, 48, 51, 52] and dystonic [49])
- Leukoencephalopathy [53, 54]
- Laryngeal innervation problems [24, 55]
- Cerebral infarction [48]
- Seizures [31, 56, 57]
- Optic neuropathy [58] or other visual impairment [31, 39, 41, 43]

In the majority of these cases, on direct questioning, the patients also reported more typical symptoms of B12 deficiency either prior to or concurrent with their rarer presentations.

Other case reports have described the co-occurrence of PA with other autoimmune and neurological diseases, including myasthenia gravis and Lambert Eaton myasthenic syndrome [59, 60]; larger case series show that PA patients are at increased risk of developing the neurological conditions multiple sclerosis, Parkinson's disease and neuromyelitis optica [61–63].

There are well-recognised associations between PA and other autoimmune conditions, particularly autoimmune thyroid disease [64–66].

Psychiatric/Cognitive Involvement and Dementia

Psychiatric and cognitive symptoms have been described in some of the case series discussed above, as well as in numerous case reports. The rate of involvement tends to be somewhat less than the neurologic signs and symptoms, with "mental impairment" being described in 12 % of episodes in Healton et al.'s large study [41] and memory loss in one-third of non-anaemic B12-deficient patients [40], although Holmes described cerebral involvement in 56 % of his patients with other nervous system involvement [31] and memory loss and poor concentration were reported by the majority of respondents in the PA Society online survey [42]. Depression, confusion, disorientation, personality change, delusions and even frank psychosis have been described on several occasions [25, 30–32, 40, 41, 45, 57, 67–69], and there

Table 16.2 "Top 5" psychiatric and cognitive abnormalities from large case series

Study	Details of subjects	Psychiatric manifestation	% age patients affected
Woltmann (1918)	150 PA cases	Apathy and somnolence	28
		Irritability	9.6
		Memory defects	7.2
		Depression	3.2
		Emotional instability	3.2
Holmes (1956)	25 B12-deficient patients with nervous system involvement	Slowing mental processes	56
		Confusion and memory defect	56
		Depression	28
		Delusions	20
		Hallucinations	12
Shorvon (1980)	50 B12-deficient patients	Organic change	26
		Affective change	20
Lindenbaum (1988)	40 non-anaemic B12-deficient patients	Memory loss	32.5
		Psychiatric disorders	17.5
		Disorientation	7.5
		Obtundation	2.5
Stabler (1990)	145 B12-deficient patients	Memory loss	13.1
		Impaired recall	6.9
		Confusion	5.5
		Disorientation	4.8
		Personality change	0.7
Healton (1991)	369 B12-deficient patients	Mental impairment	4.6
		Memory loss	1
		Paranoid psychosis	0.5
		Personality change	0.3
Hooper (2014)	889 PA patients from online survey	Memory loss	78
		Poor concentration	75
		Confusion	62
		Nominal aphasia	50

are also case reports of catatonia being due to B12 deficiency [70, 71]. As with other neurological symptoms, it has been commented upon that the cerebral manifestations are often unrelated to the degree of anaemia [31].

Table 16.2 reports the "top 5" (where available) psychiatric and cognitive abnormalities reported in large case series.

There have been several studies carried out investigating the role of B12 in cognitive impairment/dementia and B12 deficiency remains on the list of "reversible dementias" memorised by medical students, although the validity of this has been contested by a review looking at over 5,000 patients and showing that less than 1 %

had any improvement in their dementia symptoms following identification and treatment of B12 deficiency [72].

In patients with cognitive dysfunction, B12 levels in two studies were lower than in controls [73, 74]. A serial study in patients with Alzheimer's dementia showed that decline in cognitive function over time was associated with rising homocysteine levels but not with falling B12 levels [48], and another study in Alzheimer's patients showed that their B12 levels fell significantly over time [75].

There remains little evidence that B12 supplementation improves cognition in demented/memory-impaired patients [30, 48, 52, 76], although there are a few reports of improvement with therapy [67, 68], suggested to be due primarily to a shorter duration of symptoms (ideally less than 6 months) [77].

Optic/Visual Problems

Optic neuropathy due to PA has been reported in several case series [31, 58, 78]. One group suggested that it is clinically indistinguishable from tobacco amblyopia, which condition is associated with low B12 levels, even compared to non-amblyopic smoking patients [78]. Patients with PA/low B12 (even without anaemia) and SACD or other neurologic involvement but without overt visual symptoms have also been shown to have abnormal evoked potentials [79–81].

Autonomic Involvement

In early reports, there were some mentions of autonomic involvement, particularly in terms of thermoregulation, which was said to be abnormal in all patients [24]; the description of "distressing dizziness and now-and-then fainting spells" [39] could be due either to severe anaemia or to orthostatic hypotension.

In the case series reported above, impotence and urinary/faecal incontinence or urgency are frequently noted (albeit usually in a minority of cases) [21, 23, 24, 30, 41]; however, it is difficult to know whether this is purely autonomic or is more likely related to spinal cord pathology.

Orthostatic hypotension/dizziness is noted in Healton's large case series, affecting two patients [41], and the recent questionnaire study describes 59 % of PA patients as feeling "dizzy" [42]. Case reports also mention features including bronchospasm [69, 82], cardiac dysrhythmias [82] and gastric paresis [83].

More objective evidence has been produced in recent years showing that there is, not infrequently, autonomic neuropathy in PA/B12-deficient patients, who demonstrate significantly impaired orthostatic responses compared to controls, which in one study were shown to be similar to those responses seen in patients with diabetic autonomic neuropathy [84–86] and lower sympathetic and parasympathetic control of heart rate variability [85].

Investigations

Measuring B12 Deficiency

Measuring B12 on the basis of a finding of macrocytic anaemia is insufficient. Many patients with neurological signs and symptoms due to B12 deficiency will not have anaemia or macrocytosis [45, 87]. Furthermore, while the majority of blood films will show hypersegmented neutrophils, these are frequently missed on routine laboratory analysis. A significant proportion of patients with PA will also have normal or even low (due to coincident iron deficiency) MCV (mean cell volume) and haemoglobin levels; even patients with undetectable B12 levels have been reported to have normal haematologic profiles [8].

Currently, there remains debate about reference ranges, and a clinically "normal" level for serum B12 is not entirely clear, differing according to laboratory, "resulting in an inability of definitive definitions for clinical and subclinical deficiency states" [88].

The assay of B12 itself can be problematic – there are reports that high levels of IF antibodies can cause a false high B12 reading with certain assays [89, 90]. Furthermore, patients with normal (albeit low-normal) B12 levels can still have a clinical picture of deficiency which responds to B12 therapy. It has been suggested that the serum B12 levels only fall when the tissue B12 levels are severely depleted [91]. Such cases have raised interest in the testing for serum B12 metabolites [45], primarily methylmalonic acid and total homocysteine, both of which accumulate in B12 deficiency [92] and, in some cases, when the serum levels of B12 are "normal" but the patient is presenting with a B12-deficient picture [93]. These metabolites are thought to be particularly useful in representing a "tissue" rather than a "serum" B12 deficiency [94] – although it is also important to be aware that there are other causes of elevation in homocysteine (e.g. folate deficiency, renal impairment, B6 deficiency, hypothyroidism) and methylmalonic acid (older age, renal impairment).

Autoantibodies

Anti-parietal cell and anti-intrinsic factor antibodies can help in the diagnosis of autoimmune PA, but they are not infallible – one study showed that nearly 8 % of the general population tested positive for parietal cell antibodies [95]; another study showed that over 90 % of PA patients had parietal cell antibodies at diagnosis, but this fell by nearly 10 % after around six years [96]; a third study showed that only 55 % of PA patients tested positive for parietal cell antibodies [97].

Antibodies against intrinsic factor also vary depending on the study – one showing positivity in 70 % of patients [97] and another showing positive antibodies in nearly 40 % of patients at diagnosis, rising to nearly 60 % by 6 years [96].

Neurophysiological Investigations

Nerve conduction studies frequently (well over 50 %) show abnormalities in PA/B12-deficient patients, even those without neurological signs/symptoms and at all stages of the disease. Both axonal and mixed axonal/demyelinating pictures have been described [41, 43, 98]. It has been estimated that around 8 % of undiagnosed peripheral neuropathy cases may be due to B12 deficiency [99]. Some studies have also compared central and peripheral nerve conduction, usually showing more marked central than peripheral involvement [75, 88, 93].

MRI

Imaging findings largely reflect the recognised pathology identified a century or more ago.

The classical finding on spinal MRI is increased signal on T2-weighted images in the posterior columns of the cervical and/or thoracic cord [6, 73, 100, 101]. However, depending on the duration, extent and severity of the disease, one may also see involvement of the lateral and anterior columns, cord swelling or cord atrophy [73, 101, 102].

Brain lesions have also been identified on MRI, again, typically showing non-specific high-signal foci on T2-weighted images, reported to be distributed in various areas, including the cerebral hemispheric white matter, medulla oblongata, pons and mesencephalon [6, 100]. Interestingly, in certain subgroups, B12 levels have been associated with white matter lesion load [103].

See Fig. 16.1 for examples in MRI scans showing T2 hyperintensity in the dorsal columns bilaterally and in the right cord. (Images kindly provided by Dr R Dineen, Clinical Associate Professor of Neuroimaging, University of Nottingham.)

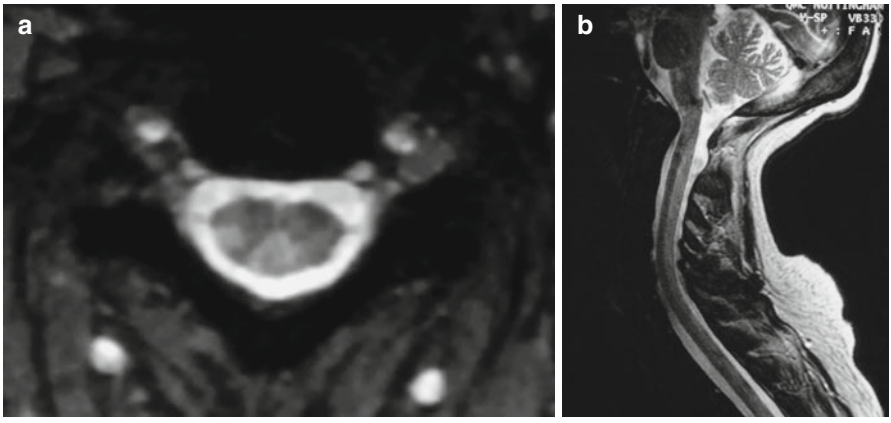

Fig. 16.1 (**a**, **b**) MRI scans showing T2 hyperintensity in the dorsal columns bilaterally and in the right cord (Images kindly provided by Dr R Dineen, Clinical Associate Professor of Neuroimaging, University of Nottingham)

Current Treatment Recommendations

UK recommendations for patients with B12 deficiency-induced neurologic impairment are for hydroxocobalamin 1 mg intramuscularly on alternate days until no further improvement and then a further 1 mg every 2 months [104]. Neurologic impairment mandates immediate treatment on diagnosis, parenteral administration and higher doses than those used for pure haematological manifestations, given under close supervision [88, 105]. A diagnosis of PA indicates treatment must be given for life.

Responses to Treatment

Patients report a subjective sense of improvement within hours of starting treatment, with haematologic abnormalities returning to normal within around 2 months (although homocysteine and methylmalonic acid tend to normalise earlier, within the first week of treatment [88]) and neurologic improvement starting within a few weeks and usually continuing over 2–12 months usually [8, 88]. It has been reported that paraesthesiae often respond early, even within a matter of days, whereas spasticity and bladder involvement take longer to respond to B12 therapy [41].

Many case series and [69]reports described total or near total resolution of neuropsychiatric signs and symptoms, improvement/total normalisation of MRI abnormalities in brain and spinal cord, normalisation of EEG abnormalities and improvement in deranged orthostatic responses following appropriate B12 treatment [6, 47–50, 53–55, 57, 59, 67, 68, 70, 71, 73, 82–84, 100–102, 106–108]. In some cases, stabilisation and prevention of further deterioration is the aim – for instance, in established visual loss or cognitive impairment, where it is thought that significant axonal loss has already occurred [58, 68]; this corresponds with findings that NCS abnormalities commonly do not resolve with B12 therapy but, unlike other neuropathies, fail to deteriorate further [98, 99]. Interestingly, impaired central nerve conduction has been shown to improve with B12 treatment, in contrast to peripheral nerve conduction [75].

These case reports correspond mostly to the larger series available in the literature in terms of recognised responses to treatment. Cognitively, although one study showed improvement in 40/56 patients with dementia following 12 months of B12 treatment (albeit most marked in patients with a short duration of symptoms and not in a placebo-controlled trial [77]) and another study in dementia patients suggested that “the average decline in cognitive function by the supplemented patients was less than expected” despite not including a control group [109], a Cochrane review found that there was insufficient evidence for B12 supplementation in cognitive impairment [110].

Neuropsychiatric symptoms “disappeared or were greatly improved after B12 therapy” in 12/14 cases in one study [31] and in 5/6 cases in another, including, delightfully, the problem of being “prone to talk absolute nonsense” [69].

Studies from the past three decades mostly show significant improvement in neuropsychiatric manifestations in the majority of patients treated with B12 (ranging from 47 to 100 % between studies [40, 41, 45]), although residual abnormalities are far from uncommon, often manifesting as impaired vibration or joint position sense, paraesthesia or abnormal reflexes [40]. It has been reported that psychiatric symptoms are less likely to respond than neurologic symptoms [45], although this is contested by other studies [40, 41, 69]. On average, a patient's symptoms have been estimated to improve by 75 % and, perhaps unsurprisingly, those with more severe symptoms and a longer duration prior to starting therapy, are more likely to have residual abnormalities [41].

Conclusions and Further Questions

B12 deficiency is recognised to cause a wide variety of neuropsychiatric manifestations, the majority of which respond to appropriate treatment, if started early enough, to a greater or lesser degree. Cognitive impairment, however, has not been shown to be consistently associated with B12 deficiency, and a Cochrane review found insufficient evidence to recommend routinely using supplements in cognitively impaired patients.

Investigations such as MRI and electrophysiological testing do not show abnormalities specific to B12 deficiency, and measurement of full blood count and even B12 levels can be misleading; it is important to be able to put together the signs, symptoms and investigation results to come to the diagnosis and institute treatment rapidly.

There is a great deal of scope for further research on this topic – not only the role of B12 in cognitive impairment but also the seeming predilection for B12 deficiency to cause primarily either neurologic or haematologic problems – as pondered by Hurst, who wondered "whether a haemolysin or a neurotoxin [was] more active" [27].

We also do not yet understand how and why B12 deficiency causes disruption to myelination of the nervous system, from the cerebrum down to the peripheral nerves, and it is possible that better understanding of these mechanisms may also aid our understanding of other demyelinating diseases.

References

1. Doscherholmen A, Hagen PS. A dual mechanism of vitamin B12 plasma absorption. J Clin Invest. 1957;36(11):1551–7.
2. Beltramello A, et al. Subacute combined degeneration of the spinal cord after nitrous oxide anaesthesia: role of magnetic resonance imaging. J Neurol Neurosurg Psychiatry. 1998;64(4):563–4.
3. Flippo TS, Holder Jr WD. Neurologic degeneration associated with nitrous oxide anesthesia in patients with vitamin B12 deficiency. Arch Surg. 1993;128(12):1391–5.

4. Holloway KL, Alberico AM. Postoperative myeloneuropathy: a preventable complication in patients with B12 deficiency. J Neurosurg. 1990;72(5):732–6.
5. Singer MA, et al. Reversible nitrous oxide-induced myeloneuropathy with pernicious anemia: case report and literature review. Muscle Nerve. 2008;37(1):125–9.
6. Timms SR, Cure JK, Kurent JE. Subacute combined degeneration of the spinal cord: MR findings. AJNR Am J Neuroradiol. 1993;14(5):1224–7.
7. NHS.UK. Vitamins and minerals – vitamin B12. 2014; Available from: http://www.nhs.uk/Conditions/vitamins-minerals/Pages/Vitamin-B.aspx.
8. Pruthi RK, Tefferi A. Pernicious anemia revisited. Mayo Clin Proc. 1994;69(2):144–50.
9. Herbert V. Vitamin B-12: plant sources, requirements, and assay. Am J Clin Nutr. 1988;48(3 Suppl):852–8.
10. Grasbeck R. Hooked to vitamin B12 since 1955: a historical perspective. Biochimie. 2013;95(5):970–5.
11. Doets EL, et al. Systematic review on daily vitamin B12 losses and bioavailability for deriving recommendations on vitamin B12 intake with the factorial approach. Ann Nutr Metab. 2013;62(4):311–22.
12. Sethi N, Robilotti E, Sadan Y. Neurological manifestations of vitamin B12 deficiency. Internet J Nutr Wellness. 2004;2(1).
13. Gomes-Trolin C, et al. Influence of vitamin B12 on brain methionine adenosyltransferase activity in senile dementia of the Alzheimer's type. J Neural Transm. 1996;103(7):861–72.
14. Kozyraki R, Cases O. Vitamin B12 absorption: mammalian physiology and acquired and inherited disorders. Biochimie. 2013;95(5):1002–7.
15. Combe J. History of a case of anaemia. Transactions of the Medico-Chirurgical Society of Edinburgh. 1824. p. 194–204. http://archive.org/stream/transactionsmed00unkngoog/transactionsmed00unkngoog_djvu.txt
16. Addison T. Anaemia – disease of the suprarenal capsules. London Med Gazette. 1849;43:517–8.
17. Flint A. A clinical lecture on anaemia. Am Med Times. 1860;1:181–6.
18. Fenwick S. On atrophy of the stomach. Lancet. 1870;96(2446):78–80.
19. Fenwick S. Lecture on atrophy of the stomach. Lancet. 1877;110(2810):1–4.
20. Dana C. The degenerative diseases of the spinal cord, with the description of a new type. J Nerv Mental Dis. 1891;16(4):205–16.
21. Putnam J. A group of cases of systemic scleroses of the spinal cord, associated with diffuse collateral degeneration; occurring in enfeebled persons past middle life, and especially in women; studied with particular reference to etiology. J Nerv Ment Dis. 1891;16(2):69–110.
22. Stewart G. Notes of a case probably referable to a recently described form of spinal disease. Br Med J. 1891;1(1588):1219–20.
23. Dana C. Subacute combined sclerosis of the spinal cord and its relation to anemia and toxemia. J Nerv Ment Dis. 1899;26:1–19.
24. Russell J, Batten F, Collier J. Subacute combined degeneration of the spinal cord. Brain. 1900;23:39–110.
25. McAlpine D. A review of the nervous and mental aspects of pernicious anaemia. Lancet. 1929;2:643–7.
26. Greenfield J. Subacute combined degeneration and pernicious anaemia. Lancet. 1933;2:62–3.
27. Hurst A, Bell J. The pathogenesis of subacute combined degeneration of the spinal cord, with special reference to its connection with Addison's (pernicious) anaemia, achlorhydria and intestinal infection. Brain. 1922;45:266–81.
28. Hurst AF. An address on addison's (pernicious) anaemia and subacute combined degeneration of the spinal cord. Br Med J. 1924;1(3290):93–100.
29. Richmond J, Davidson S. Subacute combined degeneration of the spinal cord in non-addisonian megaloblastic anaemia. Q J Med. 1958;27(108):517–31.

30. Adams R, Kubik C. Subacute degeneration of the brain in pernicious anaemia. N Engl J Med. 1944;231(1):1–9.
31. Holmes JM. Cerebral manifestations of vitamin-B12 deficiency. Br Med J. 1956;2(5006):1394–8.
32. Ferraro A, Arieti S, English W. Cerebral changes in the course of pernicious anaemia and their relationship to psychic symptoms. J Neuropathol Exp Neurol. 1945;4(3):217–39.
33. Fraser TR. Bone-marrow in the treatment of pernicious anaemia. Br Med J. 1894;1(1744):1172–4.
34. Minot G, Murphy W. Treatment of pernicious anaemia by a special diet. JAMA. 1926; 87:470–6.
35. Chanarin I. Historical review: a history of pernicious anaemia. Br J Haematol. 2000;111(2):407–15.
36. Dyke S, Harvey E. The efficient treatment of pernicious anaemia. Lancet. 1933;2:59–62.
37. Vaughan J. The treatment of anaemias. Lancet. 1933;2:63–6.
38. Scott JM, Molloy AM. The discovery of vitamin B(12). Ann Nutr Metab. 2012; 61(3):239–45.
39. Woltmann H. The nervous symptoms in pernicious anemia – an analysis of 150 cases. Collected papers of the Mayo Clinic. 1918;10:933–43.
40. Lindenbaum J, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. N Engl J Med. 1988;318(26):1720–8.
41. Healton EB, et al. Neurologic aspects of cobalamin deficiency. Medicine (Baltimore). 1991;70(4):229–45.
42. Hooper M, et al. Patient journeys: diagnosis and treatment of pernicious anaemia. Br J Nurs. 2014;23(7):376–81.
43. Shorvon SD, et al. The neuropsychiatry of megaloblastic anaemia. Br Med J. 1980;281(6247):1036–8.
44. Stabler SP. Vitamin B12 deficiency in older people: improving diagnosis and preventing disability. J Am Geriatr Soc. 1998;46(10):1317–9.
45. Stabler SP, et al. Clinical spectrum and diagnosis of cobalamin deficiency. Blood. 1990;76(5):871–81.
46. Gamstorp I, Kupfer C. Denervation of extraocular and skeletal muscles in a case of pernicious anemia. Neurology. 1961;11:182–4.
47. Kandler RH, Davies-Jones GA. Internuclear ophthalmoplegia in pernicious anaemia. BMJ. 1988;297(6663):1583.
48. Shyambabu C, et al. Serum vitamin B12 deficiency and hyperhomocysteinemia: a reversible cause of acute chorea, cerebellar ataxia in an adult with cerebral ischemia. J Neurol Sci. 2008;273(1–2):152–4.
49. Pacchetti C, Cristina S, Nappi G. Reversible chorea and focal dystonia in vitamin B12 deficiency. N Engl J Med. 2002;347(4):295.
50. Kumar S. Vitamin B12 deficiency presenting with an acute reversible extrapyramidal syndrome. Neurol India. 2004;52(4):507–9.
51. Celik M, et al. Involuntary movements associated with vitamin B12 deficiency. Parkinsonism Relat Disord. 2003;10(1):55–7.
52. Dogan EA, Yuruten B. Spinal myoclonus associated with vitamin B12 deficiency. Clin Neurol Neurosurg. 2007;109(9):827–9.
53. Heckmann JG, et al. Reversible leukoencephalopathy due to vitamin B12 deficiency in an acromegalic patient. J Neurol. 2003;250(3):366–8.
54. Vry MS, et al. Vitamin-B12-deficiency causing isolated and partially reversible leukoencephalopathy. J Neurol. 2005;252(8):980–2.
55. Ahn TB, Cho JW, Jeon BS. Unusual neurological presentations of vitamin B(12) deficiency. Eur J Neurol. 2004;11(5):339–41.
56. Kumar S. Recurrent seizures: an unusual manifestation of vitamin B12 deficiency. Neurol India. 2004;52(1):122–3.

57. Strachan RW, Henderson JG. Psychiatric syndromes due to avitaminosis B 12 with normal blood and marrow. Q J Med. 1965;34(135):303–17.
58. Larner AJ. Visual failure caused by vitamin B12 deficiency optic neuropathy. Int J Clin Pract. 2004;58(10):977–8.
59. Chang KH, et al. Coexistence of pernicious anemia and myasthenia gravis – a rare combination of autoimmune diseases in Taiwan. J Formos Med Assoc. 2006;105(11):946–9.
60. Oh SJ, Sher E. MG and LEMS overlap syndrome: case report with electrophysiological and immunological evidence. Clin Neurophysiol. 2005;116(5):1167–71.
61. Iyer A, et al. A review of the current literature and a guide to the early diagnosis of autoimmune disorders associated with neuromyelitis optica. Autoimmunity. 2014;47(3): 154–61.
62. Edwards LJ, Constantinescu CS. A prospective study of conditions associated with multiple sclerosis in a cohort of 658 consecutive outpatients attending a multiple sclerosis clinic. Mult Scler. 2004;10(5):575–81.
63. Li X, Sundquist J, Sundquist K. Subsequent risks of Parkinson disease in patients with autoimmune and related disorders: a nationwide epidemiological study from Sweden. Neurodegener Dis. 2012;10(1–4):277–84.
64. Irvine W, et al. Immunological relationship between pernicious anaemia and thyroid disease. BMJ. 1962;(5302):454–6.
65. Tozzoli R, et al. Autoantibodies to parietal cells as predictors of atrophic body gastritis: a five-year prospective study in patients with autoimmune thyroid diseases. Autoimmun Rev. 2010;10(2):80–3.
66. Zulfiqar A, et al. Association pernicious anemia and autoimmune thyroiditis: a case-report and review of literature. Eur Geriatr Med. 2014;6(4):360–1.
67. Abramsky O. Common and uncommon neurological manifestations as presenting symptoms of vitamin-B12 deficiency. J Am Geriatr Soc. 1972;20(2):93–6.
68. Moretti R, et al. Is isolated vitamin B12 deficiency a sufficient causative factor of dementia? Eur J Neurol. 2001;8(1):87–8.
69. Smith AD. Megaloblastic madness. Br Med J. 1960;2(5216):1840–5.
70. Berry N, Sagar R, Tripathi BM. Catatonia and other psychiatric symptoms with vitamin B12 deficiency. Acta Psychiatr Scand. 2003;108(2):156–9.
71. Jauhar S, et al. Pernicious anaemia presenting as catatonia without signs of anaemia or macrocytosis. Br J Psychiatry. 2010;197(3):244–5.
72. Hector M, Burton JR. What are the psychiatric manifestations of vitamin B12 deficiency? J Am Geriatr Soc. 1988;36(12):1105–12.
73. Locatelli ER, et al. MRI in vitamin B12 deficiency myelopathy. Can J Neurol Sci. 1999;26(1):60–3.
74. Cole MG, Prchal JF. Low serum vitamin B12 in Alzheimer-type dementia. Age Ageing. 1984;13(2):101–5.
75. Tomoda H, et al. Central vs peripheral nerve conduction. Before and after treatment of subacute combined degeneration. Arch Neurol. 1988;45(5):526–9.
76. Eastley R, Wilcock GK, Bucks RS. Vitamin B12 deficiency in dementia and cognitive impairment: the effects of treatment on neuropsychological function. Int J Geriatr Psychiatry. 2000;15(3):226–33.
77. Abyad A. Prevalence of vitamin B12 deficiency among demented patients and cognitive recovery with cobalamin replacement. J Nutr Health Aging. 2002;6(4):254–60.
78. Foulds WS, et al. The optic neuropathy of pernicious anemia. Arch Ophthalmol. 1969;82(4):427–32.
79. Karnaze DS, Carmel R. Neurologic and evoked potential abnormalities in subtle cobalamin deficiency states, including deficiency without anemia and with normal absorption of free cobalamin. Arch Neurol. 1990;47(9):1008–12.
80. Pandey S, Kalita J, Misra UK. A sequential study of visual evoked potential in patients with vitamin B12 deficiency neurological syndrome. Clin Neurophysiol. 2004;115(4):914–8.

81. Troncoso J, Mancall EL, Schatz NJ. Visual evoked responses in pernicious anemia. Arch Neurol. 1979;36(3):168–9.
82. Puntambekar P, et al. Rare sensory and autonomic disturbances associated with vitamin B12 deficiency. J Neurol Sci. 2009;287(1–2):285–7.
83. Malizia RW, et al. Ambulatory dysfunction due to unrecognized pernicious anemia. J Emerg Med. 2010;38(3):302–7.
84. Moore A, et al. Orthostatic tolerance in older patients with vitamin B12 deficiency before and after vitamin B12 replacement. Clin Auton Res. 2004;14(2):67–71.
85. Sozen AB, et al. Autonomic dysfunction in vitamin B12 deficiency: a heart rate variability study. J Auton Nerv Syst. 1998;71(1):25–7.
86. Toru S, et al. Autonomic dysfunction and orthostatic hypotension caused by vitamin B12 deficiency. J Neurol Neurosurg Psychiatry. 1999;66(6):804–5.
87. Carmel R. Pernicious anemia. The expected findings of very low serum cobalamin levels, anemia, and macrocytosis are often lacking. Arch Intern Med. 1988;148(8):1712–4.
88. Di Lazzaro V, et al. Central sensory and motor conduction in vitamin B12 deficiency. Electroencephalogr Clin Neurophysiol. 1992;84(5):433–9.
89. Hamilton MS, Blackmore S, Lee A. Possible cause of false normal B-12 assays. BMJ. 2006;333(7569):654–5.
90. Yang DT, Cook RJ. Spurious elevations of vitamin B12 with pernicious anemia. N Engl J Med. 2012;366(18):1742–3.
91. Mollin DL, Ross GI. Vitamin B12 deficiency in the megaloblastic anemias. Proc R Soc Med. 1954;47(6):428–31.
92. Bailey RL, et al. Monitoring of vitamin B-12 nutritional status in the United States by using plasma methylmalonic acid and serum vitamin B-12. Am J Clin Nutr. 2011;94(2):552–61.
93. Jones SJ, et al. Central and peripheral SEP defects in neurologically symptomatic and asymptomatic subjects with low vitamin B12 levels. J Neurol Sci. 1987;82(1–3):55–65.
94. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2(3):299–316.
95. Cabrera de Leon A, et al. Factors associated with parietal cell autoantibodies in the general population. Immunol Lett. 2012;147(1–2):63–6.
96. Davidson RJ, Atrah HI, Sewell HF. Longitudinal study of circulating gastric antibodies in pernicious anaemia. J Clin Pathol. 1989;42(10):1092–5.
97. Carmel R. Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race. Clin Exp Immunol. 1992;89(1):74–7.
98. Lockner D, et al. Peripheral nerve function in pernicious anemia before and after treatment. Acta Haematol. 1969;41(5):257–63.
99. Saperstein DS, et al. Challenges in the identification of cobalamin-deficiency polyneuropathy. Arch Neurol. 2003;60(9):1296–301.
100. Katsaros VK, et al. MRI of spinal cord and brain lesions in subacute combined degeneration. Neuroradiology. 1998;40(11):716–9.
101. Pittock SJ, Payne TA, Harper CM. Reversible myelopathy in a 34-year-old man with vitamin B12 deficiency. Mayo Clin Proc. 2002;77(3):291–4.
102. Karantanas AH, Markonis A, Bisbiyiannis G. Subacute combined degeneration of the spinal cord with involvement of the anterior columns: a new MRI finding. Neuroradiology. 2000;42(2):115–7.
103. Hickie I, et al. Vascular risk and low serum B12 predict white matter lesions in patients with major depression. J Affect Disord. 2005;85(3):327–32.
104. Society R.P. British National Formulary, Hydroxocobalamin. 2014 [cited 12 Sept 2014].
105. Beck WS. Neuropsychiatric consequences of cobalamin deficiency. Adv Intern Med. 1991;36:33–56.
106. Halliday AW, Vukelja SJ. Neurologic manifestations of vitamin B-12 deficiency in a military hospital. Mil Med. 1991;156(4):201–4.

107. Duprez TP, et al. MRI of the spine in cobalamin deficiency: the value of examining both spinal cord and bone marrow. Neuroradiology. 1996;38(6):511–5.
108. Hirata A, et al. Subacute combined degeneration of the spinal cord concomitant with gastric cancer. Intern Med. 2006;45(14):875–7.
109. Kwok T, et al. Vitamin B(12) supplementation did not improve cognition but reduced delirium in demented patients with vitamin B(12) deficiency. Arch Gerontol Geriatr. 2008;46(3):273–82.
110. Malouf R, Areosa Sastre A. Vitamin B12 for cognition. Cochrane Database Syst Rev. 2003(3):CD004326.

Chapter 17
The Impact of Multiple Sclerosis on Gastrointestinal System Function

David J. Levinthal and Klaus Bielefeldt

Abstract MS patients commonly experience symptoms related to dysregulated gastrointestinal function, and these problems contribute to significant impairment in quality of life. Oropharyngeal dysphagia and anorectal dysfunction have traditionally garnered the most attention due to their more obvious impacts on daily functions. For example, convergent evidence suggests a prevalence of oropharyngeal dysphagia in 25–40 % of all MS patients. Similarly, anorectal dysfunction is quite common with ~40 % of MS patients reporting constipation and ~25 % reporting frequent fecal incontinence. In addition, many MS patients experience mixed forms of anorectal dysfunction with both constipation and fecal incontinence. There are a diverse range of potential pathophysiological mechanisms that contribute to these problems, including general impairments in skeletal motor function that are typically experienced by MS patients. However, recent research has revealed that gastrointestinal symptoms in the MS population are not limited to oropharyngeal dysphagia and anorectal dysfunction, but include dyspepsia and abdominal pain. The latter associations may reveal a broader impact of MS disease beyond impairments in skeletal motor function to include disruptions in the central neural regulation of autonomic and/or sensory processing. Despite the significant impact of gastrointestinal dysfunction on MS patient quality of life, there remains a paucity of published literature on therapeutic options for these disorders in this patient population. Thus, there is a compelling need to develop effective treatment options that should translate into improved patients' quality of life. Collaborative work between neurologists and gastroenterologists will have the best chance to advance the field and to optimize the care of MS patients suffering from symptoms related to impaired gastrointestinal function.

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Introduction

The complex and integrative functions of the gastrointestinal (GI) system require a fine coordination of skeletal muscle movements, sensory feedback, and autonomic nerve activity, all of which are influenced by the central nervous system. Thus, it is not surprising that impairments in GI system function are frequently experienced by patients with central nervous system disorders such as multiple sclerosis (MS). However, MS does not have a uniform presentation nor is it a static disorder. The specific location and extent of neuroinflammation and neuronal dysfunction varies greatly between patients and even within an individual patient over time. Therefore, the various impacts of MS disease on gastrointestinal function are inherently variable between patients and even within single individuals over time. Yet, despite this heterogeneity in the potential physiological impacts of MS disease, there are some general patterns of GI-related symptoms that many MS patients experience during the course of their illness. The aim of this chapter is to review what is currently known about the nature and prevalence of some of the more common gastrointestinal symptoms observed in MS disease. The discussion will also focus on contributing pathophysiological mechanisms and the unique management challenges posed by several of these problems. While the focus of our discussion is on MS, many of the problems described and their treatment approaches are relevant for other diseases of the central and/or peripheral nervous system that also impact GI function in patients with impaired sensory, motor, and/or cognitive function.

Prevalence of Gastrointestinal Symptoms in Multiple Sclerosis

The classical evaluation and treatment of patients with multiple sclerosis placed an emphasis on skeletal muscle function and the impact of the disease on mobility. Yet, impairments of skeletal muscle function not only affect mobility, but can compromise swallowing (deglutition), urination, and defecation. The normal processes of ingestion and elimination require highly coordinated patterns of voluntary skeletal muscle activity as well as reflexive activity that are collectively integrated within the spinal cord, brainstem, and higher-order neural systems. Thus, deglutition and elimination processes can be quite sensitive to even subtle disruption in such neural regulation. Because impairment of deglutition and elimination are common in multiple sclerosis patients [22] and contribute to poor quality of life, these symptoms have become recognized as important markers of MS disease. For at least the past

25 years, impairments in swallowing, urination, and defecation have been incorporated into widely used patient assessment instruments, such as the expanded disability status scale (EDSS) [26].

How Common Are Problems with Deglutition in Multiple Sclerosis Patients?

The specific answer reflects the methodology used to assess oropharyngeal dysphagia, the predominant form of dysphagia experienced in this population. These study methods range from standardized symptom questionnaires to more direct, objective methods such as fiberoptic visualization endoscopic evaluations of swallowing (FEES), electrophysiological study of swallowing (EPSS), or dynamic fluoroscopic methods such as videofluoroscopic swallowing study (VFSS) using contrast radiography. Distinguishing the methodology of assessment is important because patients may experience symptoms of dysphagia with or without objective abnormalities in the motor domain [53]. For example, subtle sensory abnormalities of input from the mouth, tongue, and posterior pharynx may lead to changes in the perceived timing of bolus movement during swallowing that could drive symptom reporting, even if the overall motor pattern is largely intact as assessed by the currently accepted standard methods. Dysphagia prevalence may also vary in different MS disease subgroups, such as those with primary progressive disease versus relapsing remitting disease [9], or in patients with differing MS disease severity [12]. In this context, it is not surprising that the published literature shows a range of prevalence estimates for dysphagia in large groups of MS patients with heterogeneous mixes of disease subtypes and severity. A review of 11 studies [1, 2, 4, 5, 9, 11, 12, 21, 27, 39, 49] that used either symptom questionnaires and/or objective visualization measures to assess dysphagia shows that the prevalence of swallowing disorders among patients with multiple sclerosis is likely between 25 and 40% (Fig. 17.1a). As shown in Fig. 17.1a, the variability in prevalence estimates is high for small studies, but converges to a smaller range in studies with larger numbers of study participants. Although the reported data may be skewed due to tertiary referral center bias or failure to stratify for disease subtypes or MS symptom severity, it nonetheless demonstrates the potential importance of this often underappreciated problem.

What Is the Prevalence of Anorectal Dysfunction in Multiple Sclerosis Patients?

Anorectal dysfunction is a common complaint among patients with MS. Patients with MS commonly have a variety of impairments in the strength and recruitment of pelvic floor muscles that are critical for coordinating the timing and efficacy of

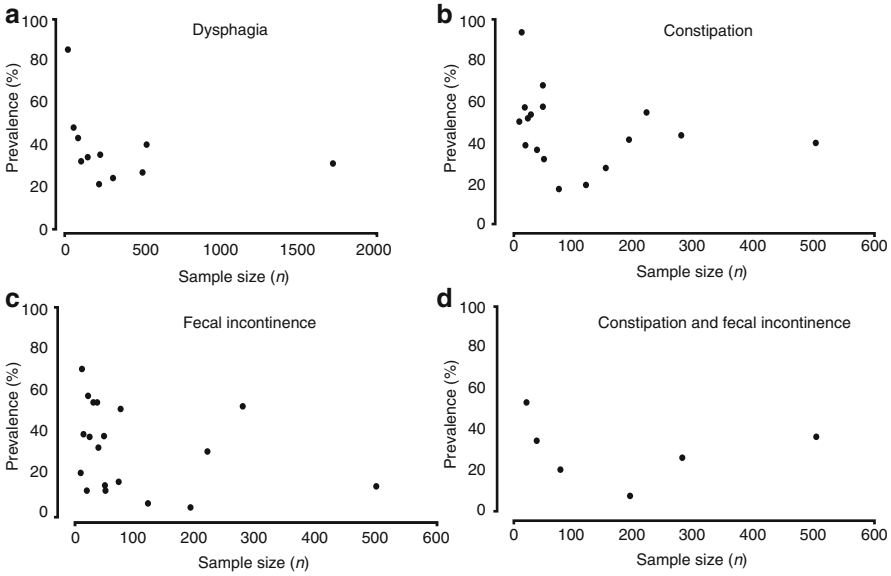

Fig. 17.1 Prevalence estimates for dysphagia (**a**), constipation (**b**), fecal incontinence (**c**), and mixed constipation and fecal incontinence (**d**) in patients with MS

defecation, as well as for maintaining fecal continence. Furthermore, impaired colonic motility may result from either a direct impact of MS disease, or as a side effect of medications used to alleviate other MS-related symptoms, and contribute to problems with defecation. Finally, the initiation of defecation requires effective straining, which can also be impaired as the illness advances to include ineffective recruitment of abdominal wall muscles needed to increase intraabdominal pressure. Collectively, *anorectal dysfunction* or *bowel dysfunction* are general clinical terms that incorporate symptoms of constipation or fecal incontinence. Constipation and fecal incontinence can exist in isolation or occur together. As with the studies of dysphagia in MS patients, there is a range of published prevalence estimates for constipation and fecal incontinence in this population. This is again likely due to differences in study methodology, as different studies use varying definitions of constipation and/or fecal incontinence. Most prevalence studies have used general consensus criteria and a combination of patient self-assessments and validated scoring systems. Some additional studies incorporate physiological markers (such as whole gut transit time assessed with Sitz markers) in combination with reported symptoms. We recently examined the published literature on the prevalence of anorectal dysfunction in the multiple sclerosis population [35]. As shown in Fig. 17.1b, our review reported a range of prevalence estimates for constipation across 17 studies (cited in [35]). However, studies with larger populations generally converged with estimates close to 40 %, which thus represents the best estimate for the true prevalence of constipation in MS patients and is consistent with the clinical experience of the authors. The prevalence of fecal incontinence in MS patients is lower

than constipation. As seen in Fig. 17.1c, our same review reported prevalence estimates of fecal incontinence in MS patients from 19 studies (cited in [35]), most of which were smaller studies involving fewer than 50 patients. Therefore, there was significant variability in the estimate of symptom prevalence of fecal incontinence in this population. In studies with greater than 100 MS patients, the range of estimates was still wide-ranging, from 3.4 to 51 % [35]. It is generally accepted that the typical prevalence of fecal incontinence in the general population is on the order of ~10 % [7]. Despite the described shortcomings of published studies, the aggregate data clearly indicate a higher prevalence with ~25 % of MS patients experiencing fecal incontinence. Finally, the prevalence of the mixed form of anorectal dysfunction (i.e., patients experience both constipation and fecal incontinence) is less clear, with a wide range of estimates from between 6 and 52 % of MS patients (Fig. 17.1d) [35]. Although mixed forms of anorectal dysfunction are likely less common than the isolated occurrence of constipation or incontinence, this population of MS patients constitutes an important subgroup that poses significant therapeutic challenge for clinicians.

The impact of multiple sclerosis on gastrointestinal function may not be limited to impairments in skeletal muscle coordination, and historically, this possibility has received less attention. Depending upon the specific location of MS lesions and the severity of neuroinflammation, there could be a range of potential disruptions to the central neural circuits that govern sensation and autonomic regulation. Such central neural circuit disruption could directly generate gastrointestinal symptoms beyond the aforementioned difficulties with deglutition and defecation. However, the range of GI symptoms typically experienced by MS patients has not been well established. To address this gap in knowledge, we recently conducted a large, comprehensive survey to assess the extent and prevalence of GI symptoms in MS patients [27]. In this study, the validated Rome III questionnaire was used to assess the prevalence of GI symptoms in a single center cohort of 218 patients with MS disease. Our analysis showed that the majority of patients (66 %) experienced at least one chronic GI symptom. Not surprisingly, we reconfirmed the presence of dysphagia (21 %), constipation (37 %), and fecal incontinence (15 %) in our cohort, with prevalence estimates that generally correspond to other studies (see Fig. 17.1). However, we also discovered a fairly high prevalence of dyspepsia (28 %) and abdominal pain (14 %), symptoms which are not traditionally regarded as being associated with MS (Fig. 17.2). Dyspepsia incorporates symptoms of early satiety, postprandial fullness, and epigastric discomfort and points toward dysfunction in the sensory and motor function of the stomach. The neural mechanisms that integrate these functions are distinct from those that drive impaired skeletal muscle coordination, and therefore MS patients may have dyspepsia but no dysphagia or anorectal dysfunction. Further evidence for disruption in the normal motility or sensory function of the GI tract in MS patients is the significant number of patients that reported bloating, belching, and nausea (Fig. 17.2). Thus, patients with MS may suffer from a variety of GI symptoms, and these symptoms are not constrained to mechanisms dependent upon impaired control of skeletal muscle function, but rather may involve more global dysfunction of sensory and autonomic regulation.

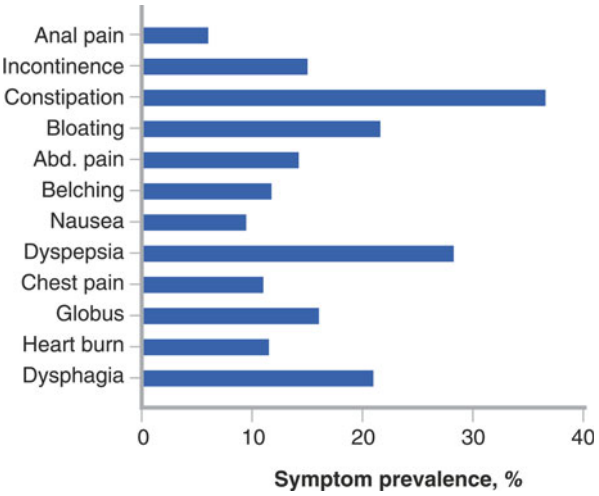

Fig. 17.2 Prevalence of gastrointestinal symptoms in a single center cohort of 218 MS patients as assessed by the Rome III questionnaire (Modified from Levinthal et al. [27])

Pathophysiological Mechanisms and Treatments for MS-Associated Dysphagia

Swallowing involves the successful manipulation and propulsion of ingested material from the oral cavity into the stomach while simultaneously preventing material from entering the proximal airway. Although swallowing can be triggered volitionally, many aspects of the process rely on highly coordinated patterns of activity among dozens of striated muscles within the oropharynx and proximal esophagus. This aspect of swallowing (deglutition) is referred to as the *oropharyngeal phase*, and disruption in this phase defines *oropharyngeal dysphagia*. Swallowing also requires the coordination of skeletal and smooth muscle activity within the tubular esophagus and sphincteric structures. This latter aspect of swallowing is referred to as the *esophageal phase*, and disruption in this phase defines *esophageal dysphagia*. The precise interplay of muscle movements that support normal swallowing requires intact sensory, motor, and autonomic nerves, with appropriate reflexive integration within the central nervous system. Hence, the symptom of dysphagia can result from any disturbance within these varied sensorimotor and autonomic systems. MS patients are particularly vulnerable to developing dysphagia given the likelihood that the disease causes neural dysfunction within one or more of the distributed CNS sites required to coordinate optimal swallowing. However, normal esophageal peristalsis that propels an ingested bolus along the smooth muscle portion of the esophagus is largely preprogrammed by the enteric nervous system. As enteric neurons are not directly affected by a central demyelinating process, most MS patients experience oropharyngeal dysphagia rather than esophageal dysphagia [39, 48]. Although many clinical studies have demonstrated an association of severe oropharyngeal dysphagia with more severely progressed MS-related disability [9, 12, 39], even patients with mild or moderate MS disease severity can experience some degree of oropharyngeal phase impairments in swallowing [39].

MS-related dysphagia remains an important and active area of research because the clinical consequences of untreated dysphagia can be severe. Beyond its potential impact on nutrition, oropharyngeal dysphagia carries an inherently high risk of aspiration pneumonia if left unaddressed. Indeed, some MS patients with severe oropharyngeal dysphagia ultimately require long-term restrictions on oral intake to minimize the likelihood of aspiration. While behavioral modifications, food choices, and manipulations of food consistency potentially mitigate this risk, there are few effective therapies to improve the true underlying problem. Prolonged oral restriction may require the placement of a percutaneous gastrostomy (PEG) tube. While PEG tube placement may ensure adequate enteral alimentation, it can significantly interfere with patient quality of life. We recently reviewed patterns of hospitalization and treatments in MS patients. We found that slightly less than 1 % of all MS-related hospital admissions were associated with the placement of a gastrostomy tube, which was presumably performed due to patient difficulties with oral intake in the context of oropharyngeal dysphagia [36]. Unfortunately, the rate of gastrostomy tube placement remained stable between 2001 and 2010, despite the advent and widespread adoption of disease-modifying agent use over this time period [36]. Thus, the cumulative clinical impact of oropharyngeal dysphagia on important clinical outcomes (gastrostomy tube placement, aspiration pneumonia, etc.) and associated impairments in MS patient quality of life continues to increase.

Ongoing research continues to define the precise locations of CNS sites that are required to support optimal swallowing. Several brainstem nuclei are known to contain the motor neurons that directly influence the muscles of the tongue, epiglottis, and pharynx. Thus, it is not surprising that MS patients with documented brainstem lesions suffer disproportionately from oropharyngeal dysphagia [2, 9]. However, disruption of either sensory inputs and/or descending commands from higher brain sites to these brainstem nuclei can also lead to oropharyngeal dysphagia. For example, impaired integration in the brainstem as assessed by delayed or absent gag reflex is associated with dysphagia in MS patients [53]. Similar mechanisms may link the presence of an impaired gag reflex with impairments in the protective cough reflex, and thus MS patients with brainstem lesions may be at especially high risk to develop “silent” aspiration. At the cerebral cortical level, multiple sites have been implicated in the regulation of tongue and pharyngeal muscle contractions that support the act of swallowing [19, 24, 38]. These cortical sites include regions of the lateral frontal operculum [38] as well as more rostromedial regions of the lateral hemispheres that span premotor areas and the anterior motor cortex [19]. Most people have an asymmetric, bilateral representation of the pharyngeal muscles, with one of these representations dominating pharyngeal control [19, 20]. Disruption in neural activity within the dominant pharyngeal cortical control region is sufficient to induce oropharyngeal dysphagia, even in normal individuals [24]. Interestingly, the cortical systems that regulate swallowing are capable of undergoing significant neuroplastic changes. For example, strokes involving the dominant motor cortical representation of the pharynx can lead to dysphagia, and dysphagia recovery is associated with an increase in the areal distribution and increase in the cortical excitability within the non-lesioned, previously non-dominant hemispheric

Table 17.1 Mechanisms contributing to dysphagia in MS patients

Citation	Study population	Objective testing	Swallowing dysfunction
Thomas and Wiles [49]	79 hospitalized MS patients	Physical exam Bedside swallow testing	Weak jaw, neck, and tongue Delayed water swallow
Abraham and Yun [2]	13 MS patients	Videofluoroscopy	Epiglottic dysmotility Pharyngeal constrictor dysfunction (85 %)
Calcagno et al. [9]	49 primary and secondary progressive MS patients with dysphagia	Bedside swallow testing FEES[a]	Impaired tongue movements (92 %) Laryngeal and epiglottic dysmotility (57 %) Soft palate dysmotility (69 %)
Wiesner et al. [53]	18 MS patients	Videofluoroscopy	Laryngeal and epiglottic dysmotility (61 %) Frank aspiration (22 %)
Alfonsi et al. [3]	26 MS patients	FEES[a] EPSS[b]	Laryngeal and pharyngeal dysmotility (54 %) Abnormal tongue EMG (65 %) Abnormal laryngeal-pharyngeal EMG (65 %) Fewer cricopharyngeal EMG pauses (31 %)

[a]Fiberoptic endoscopic evaluation of swallowing (FEES)
[b]Electrophysiological study of swallowing (EPSS)

representation [20]. Indeed, repetitive transcranial magnetic stimulation (rTMS) directed to the contralesional pharyngeal motor cortex is capable of increasing cortical excitability within the region and is associated with improvements in both poststroke dysphagia symptoms and objective measures of swallowing function [37]. This important clinical observation suggests that rTMS therapy to the contralesional pharyngeal motor cortex in MS patients may be developed as a viable therapy to reduce MS-related oropharyngeal dysphagia that is not clearly related to brainstem disease.

The published literature that details specific, objective measures of swallowing function in MS patients is quite sparse (Table 17.1). These methods include bedside swallowing evaluations, fiberoptic visualization endoscopic evaluation of swallowing (FEES), videofluoroscopic swallowing studies (VFSS), and electrophysiological assessments [2, 3, 9, 49, 53]. Despite being fairly small clinical studies with heterogeneous groups of MS patients, there are several common findings. These studies demonstrate a range of tongue, laryngeal, epiglottic, soft palate, and pharyngeal muscle impairments, even in MS patients without symptomatic oropharyngeal dysphagia. Interestingly, some of these muscles demonstrate spastic changes rather than weakness. For example, a subset of MS patients experience dysphagia associ-

ated with impaired relaxation of the cricopharyngeal muscle, which is a major contributor to the upper esophageal sphincter (UES). This latter observation may be particularly relevant for oropharyngeal dysphagia therapy, as such abnormalities can be objectively determined with appropriate diagnostic studies to identify a subset of patients with a high likelihood to respond to therapies directed at the cricopharyngeal muscle.

There are no current systemic pharmacological treatments for MS-related oropharyngeal dysphagia. Typically, therapeutic interventions in patients with oropharyngeal dysphagia of any cause include functional swallowing therapies that include altering the diet to accommodate the degree of dysfunction (i.e., thickening liquids), methods to compensate or adapt to the dysfunction (i.e., tucking the chin while swallowing to passively direct material to the esophagus and close off the upper airway; Mendelsohn maneuver with external lift to the cricoid during swallowing to reduce laryngeal movement), and exercises to attempt to maintain residual function. Only two therapeutic trials have been conducted in MS patients with oropharyngeal dysphagia. The first study investigated the efficacy of cricopharyngeal-directed botulinum toxin A injection in 14 MS patients with a documented hypertonic upper esophageal sphincter [44]. The results of this small study showed at least moderate improvement in dysphagia in all 14 patients, but the effects were relatively short-lived with a return to baseline dysfunction at 6 months following the injections. As indicated above, cricopharyngeal botulinum toxin injection would not be predicted to benefit MS patients with predominantly oral dysphagia or oropharyngeal dysphagia not associated with UES hyperactivity. A second study by the same research group investigated the ability of direct electrical pharyngeal muscle stimulation to improve oropharyngeal dysphagia in 20 symptomatic MS patients [43]. A catheter fitted with bipolar platinum ring electrodes was placed transnasally with the electrode tips located 3 cm above the UES. Patients then underwent either five consecutive daily sessions with 10 min of stimulation (75 % of pain threshold) or sham stimulation. Interestingly, the treatment group experienced a significant improvement in VFSS measures which were durable for at least 4 weeks after the final stimulation session. The mechanisms that mediate the reported treatment effect are not clear. Nevertheless, pharyngeal muscle electrical stimulation may be a promising intervention that should be further explored for the treatment of oropharyngeal dysphagia in MS patients.

Pathophysiological Mechanisms and Treatment for MS-Associated Anorectal Dysfunction

Normal defecation patterns require both conscious and unconscious sensory and motor processing within the CNS to support the perception of rectal filling, the ability to retain stool in the rectum without anal leakage, and the volitional elimination of stool without difficulty. The disruption in any of these processes leads to

anorectal dysfunction, a term that encapsulates the symptoms of constipation and/or fecal incontinence. Anorectal dysfunction is a source of significant impairment in quality of life in affected MS patients and is a source of substantial caregiver burden [35]. MS patients may experience anorectal dysfunction because even subtle impairments in sensation from the anorectum and/or the timing and efficacy of abdominal, pelvic floor, and anal muscle contractions are sufficient to generate symptoms. For example, impaired sensation of rectal filling may predispose to fecal incontinence (due to lack of perceived urge) or even fecal impaction. Altered central neural control may result in spasticity of pelvic floor muscles that can impair the ability to evacuate rectal contents easily. Additionally, it is possible that MS disease itself could directly influence colonic transit and contractility due to disruption in normal patterns of CNS influences over the autonomic regulation of the colon. Because these various sensory, motor, and autonomic functions are supported by neurons in disparate regions of the brain, brainstem, and spinal cord, there is an increased likelihood that any one MS lesion could impact some aspect of anorectal function. However, clinical features of many MS patients potentially confound the attribution of anorectal dysfunction as a direct result of an MS-related CNS lesion. For example, medications that are frequently used to alleviate other MS-related symptoms, such as urinary incontinence and hyperactive bladder, pain, muscle spasticity, or mood disorders, can lead to constipation as a side effect. Physical activity is independently linked with colonic motility through unclear mechanisms. Thus, MS patients with impaired mobility may suffer from constipation via reduced colonic transit time, and fecal incontinence may increase in frequency simply because such patients do not have time to reach the commode. Lastly, a subset of female MS patients could develop anorectal dysfunction due to the effects of prior obstetric trauma.

The extrinsic innervation of the pelvic structures involved in defecation arise from the pudendal nerves (motor function to the pelvic floor muscles and external anal canal; sensation from the genitalia, perineum, and anus), the pelvic nerves (parasympathetic innervation of the colon, rectum, and internal anal sphincter), and the hypogastric nerves (sympathetic innervation of the distal-most colon, rectum, and internal anal sphincter). These nerves are most immediately influenced by the activity in neurons contained within autonomic and motor nuclei of the lumbosacral spinal cord. A similar pattern of spinal and autonomic innervation also supports urinary function. Thus, MS patients with low spinal involvement are likely to experience anorectal dysfunction, often in conjunction with difficulties with urination [31, 41]. Ongoing research continues to help define the precise locations of supraspinal CNS neurons that are required to support optimal anorectal function. Multiple higher-order centers in the midbrain and brainstem have been shown to exert an influence over both bladder and anorectal function, including Barrington's nucleus, the periaqueductal gray, and the parabrachial nucleus, among others [13]. Ultimately, the cerebral cortex integrates social cues and context with sensory input to both consciously and unconsciously influence pelvic floor motor programs that are required to retain and/or eliminate stool. These cerebral cortical sites are widely distributed across bilateral regions located both in the medial wall, in particular, the

supplementary motor cortex and mid-cingulate cortex [25, 45], and within lateral regions that include the medial primary motor cortex [50], insula, lateral operculum, and posterior parietal cortex [45]. Because of the disparate locations of the cerebral cortical areas that are involved in pelvic floor function, it would follow that periventricular and internal capsular white matter disease associated with MS could easily interfere with tracts carrying descending commands to the pelvic floor. To date, there have been no reports investigating this possibility, although modern brain-imaging techniques such as diffusion tensor tractography would be well suited to do so. For example, similar methods have already been used to reveal abnormalities in white matter tracts that are associated with symptoms of fatigue, disrupted emotional regulation, and impaired cognition in MS patients [6, 33].

The published literature that details specific, objective measures of anorectal function in MS patients is limited. The few clinical studies that have investigated specific mechanisms that may contribute to anorectal dysfunction in MS patients have collectively employed a variety of colonic, rectal, and anal pressure-sensing devices, sensory testing, radiographic studies, and electrophysiological testing, as well as assessments of colonic motility. However, these studies have been conducted in relatively small numbers of mostly symptomatic patients (Table 17.2). Yet, what is apparent is that while no single mechanism can fully account for the experience of anorectal dysfunction in these MS patients, abnormalities are common. Importantly, many of these studies cannot determine the sufficiency or necessity of any given physiological biomarker because MS patients without anorectal dysfunction were typically excluded from analysis.

Nevertheless, several results were frequently observed in those studies that focused on MS patients with constipation and/or fecal incontinence. For example, most studies have demonstrated decreased resting anal tone and decreased volitional squeeze pressures, particularly in those patients with fecal incontinence. Sensory deficits were less prevalent, with only two studies demonstrating decreased subjective anal or rectal perception [34, 52]. One small study in advanced MS patients showed that such decreased sensation may occur at the brain level, rather than at the primary afferent or spinal level [18]. Defecographic methods demonstrated impaired puborectalis relaxation as a contributing factor to constipation [10, 15, 51]. Measures of colonic transit or colonic pressure-volume relationships showed delayed colonic transit and evidence for decreased colonic compliance. However, decreased colonic transit may be confounded by the presence of impaired evacuation. Finally, electrophysiological techniques reliably demonstrated impaired central motor latencies along with intact peripheral nerve-motor endplate latencies, consistent with an impact of CNS disease, rather than peripheral nerve injury.

There are few treatment options for MS patients with anorectal dysfunction, and clinical studies in this population are limited to only a few small, mostly uncontrolled trials (Table 17.3). These include studies using behavioral interventions and biofeedback in order to alter sphincteric function that showed some clinical benefit [30, 42, 54]. Unfortunately, these benefits were primarily restricted to those with mild disease. This observation is not surprising, as biofeedback requires intact central motor control systems that are likely to be disrupted in advanced MS patients

Table 17.2 Mechanisms contributing to anorectal dysfunction in MS patients

Citation	Symptom	Sample size	Assessment tool	Results
Guinet et al. [17]	CON	21	Anorectal manometry	Decreased rectoanal inhibitory reflex
Preziosi et al. [42]	CON and/or FI	39		No differences based on symptom patterns
Wiesel et al. [54]	CON and/or FI	13		Weak external sphincter Impaired straining
Nordenbo et al. [34]	CON and/or FI	30		Low squeeze pressure, impaired Valsalva pressures; increased rectoanal inhibitory reflex threshold; decreased rectal sensation
Sørensen et al. [46]	CON and/or FI	11		Lower sphincter pressures in women
Mathers et al. [28]	CON and/or FI	23		Decreased squeeze pressures Increased PR contraction
Weber et al. [52]	CON and FI	16		Impaired amplitude and duration of squeeze pressure
Munteis et al. [30]	CON and/or FI	52		Decreased squeeze pressure, anal inhibitory reflex, and PC
Waldron et al. [51]	CON and FI	6		Markedly reduced squeeze pressure
Preziosi et al. [42]	CON and/or FI	39	Rectoanal sensitivity	No significant differences in rectal or anal sensory thresholds
Sørensen et al. [46]	CON and/or FI	11		No significant difference compared to normal subjects
Weber et al. [52]	FI	5		Abnormal sensory threshold to rectal distention
Chia et al. [10]	CON	10		Normal rectal and anal sensory thresholds
Waldron et al. [52]	CON and FI	6		Normal rectal sensory threshold
Munteis et al. [30]	CON and/or FI	52		Normal rectal sensory threshold
Nordenbo et al. [34]	CON and/or FI	30		Reduced rectal sensory thresholds, particularly in FI
Jameson et al. [23]	FI	20		Normal rectal and anal sensory thresholds
Chia et al. [10]	CON	10	Defecography	Impaired PR relaxation
Waldron et al. [51]	CON and FI	6		Impaired PR relaxation
Gill et al. [15]	CON	11		Impaired PR and anal relaxation

Table 17.2 (continued)

Citation	Symptom	Sample size	Assessment tool	Results
Weber et al. [52]	CON and FI	16	Colonic transit or Colono-metrogram	Delayed colonic transit
Waldron et al. [51]	CON and FI	6		Delayed distal colonic transit
Chia et al. [10]	CON	7		Delayed colonic transit
Glick et al. [16]	CON	7		Increased rate of pressure rise with colonic infusion
Haldeman et al. [18]	CON or FI	3		Increased intracolonic pressure to low infused volume
Sørensen et al. [46]	CON and/or FI	11	Electrophysiology	Normal pudendal nerve terminal latency
Mathers et al. [28]	CON and/or FI	23		Decreased central motor conduction time
Swash et al. [47]	FI	12		Decreased central motor conduction time
Haldeman et al. [18]	CON or FI	3		Normal spinal, but decreased cortical evoked potentials to sensory nerve stimulation
Jameson et al. [23]	FI	20		Normal pudendal nerve terminal latency

EAS external anal sphincter, *PR* puborectalis, *PC* paradoxical contraction

Table 17.3 Treatment of anorectal dysfunction in MS patients

Citation	Treatment	Design	Sample size	Result	Response rate (%)
Preziosi et al. [42]	Biofeedback	Prospective series	39	Improved CON and FI scores	46
Wiesel et al. [54]		Prospective series	13	Patient rating of success	38
Munteis et al. [30]		Prospective series	18	Patient reported symptom improvement	44
McClurg et al. [29]	Abdominal massage	RCT	30	Increase in defecation frequency only at week 4; improved composite scores at weeks 4 and 8	–
Preziosi et al. [40]	Transanal irrigation	Prospective series	30	Improved CON and FI scores	53
Faaborg et al. [14]		Retrospective series	25	Reported improvement	40

CON constipation, *FI* fecal incontinence, *RCT* randomized controlled trial

with more severe forms of anorectal dysfunction. One study investigated various techniques of abdominal massage to improve constipation in MS patients and demonstrated a small effect size in the treatment group [29]. However, the intervention appears to be time consuming and may be difficult for MS patients with advanced disease to accomplish independently due to difficulties in positioning, dexterity, and strength. Two small studies using transanal irrigation in MS patients with anorectal dysfunction showed similar results with improvement in ~40–53 % of patients [14, 40].

A practical approach to the treatment of anorectal dysfunction must consider the dominant symptom, level of MS disability (particularly in regard to mobility and dexterity), and patient and caregiver preferences. For milder problems, some general approaches are likely to be effective. For constipation in those with retained mobility and intact sensation, changes in fiber intake or flexibly dosed osmotically active agents (e.g., polyethylene glycol, magnesium citrate, etc.) should suffice. For those with primarily fecal incontinence, bulking agents or antimotility agents (e.g., loperamide) could be quite helpful to firm stool consistency. The rationale for the latter recommendation is that looser stool consistency is the factor most highly associated with fecal incontinence in the general population [8]. These two general approaches may work for many MS patients with anorectal dysfunction, but are unlikely to be effective for those with severe mobility impairment and/or the presence of both constipation and fecal incontinence. For example, patients with severe mobility impairment or sensory deficits may easily develop fecal incontinence with standard therapies for constipation, or fecal impaction with standard therapies for fecal incontinence. In these circumstances, timed evacuations using scheduled administration of enemas or laxating rectal suppositories could be effective. Alternatively, for those with more severe fecal incontinence at baseline, the combination of timed evacuation with suppositories could be coupled with the cautious use of antidiarrheals between evacuations. The Consortium for Multiple Sclerosis Centers (CMSC) sponsored a meeting in the fall of 2011 to develop a practical treatment approach based upon expert experience to treat both mild and severe forms of anorectal dysfunction in MS patients [32]. These treatment guidelines incorporate many of the practical approaches mentioned above and were devised using input from both authors. However, the efficacy of these guidelines has remained untested.

Unaddressed Needs and Future Directions

As is clear from more recent symptom surveys, gastrointestinal dysfunction remains a very common contributor to impaired quality of life in many MS patients. Oropharyngeal dysphagia and anorectal dysfunction have traditionally garnered the most attention, perhaps because of their more obvious, daily impact on eating and defecation. However, treatment options and efficacy for these problems remain limited, and more research is needed to optimize the care of MS patients with gastrointestinal dysfunction. In a fragmented healthcare delivery system, the

reciprocal relationship between many neurological disorders with gastrointestinal symptoms constitutes an important clinical challenge. Treatment choices must not only target the underlying gastrointestinal abnormality, but also consider the often significant neurological deficits that may influence treatment results and feasibility. While neurological illnesses such as MS directly affect many other body systems as outlined above, understanding this interdependence also requires healthcare providers to understand treatments and their side effects, as many of the medical interventions for neurological illness inherently alter gut function. Several other gastroenterological symptoms are just beginning to be recognized in MS patients. More research is needed to quantify the impact of specific symptoms on disease-related quality of life, as this will help prioritize future clinical studies and the development of treatment options. For example, dyspepsia is surprisingly common in MS patients and is associated with significant impairment in quality of life. However, the mechanisms that drive this symptom are not clear. Future research should work to uncover contributing mechanisms that drive dyspeptic symptoms in MS patients, as well as evaluate the efficacy of treatments. Collaborative work between neurologists and gastroenterologists will have the best chance to advance the field and optimize the care of MS patients that suffer from impaired gastrointestinal function.

References

1. Abraham S, Scheinberg LC, Smith CR, LaRocca NG. Neurologic impairment and disability status in outpatients with multiple sclerosis reporting dysphagia symptomatology. J Neurol Rehab. 1997;11:7–13.
2. Abraham SS, Yun PT. Laryngopharyngeal dysmotility in multiple sclerosis. Dysphagia. 2002;16:69–74.
3. Alfonsi E, Bergamaschi R, Cosentino G, Ponzio M, Montomoli C, Restivo DA, Brighina F, Ravaglia S, Prunetti P, Bertino G, Benazzo M, Fontana D, Moglia A. Electrophysiological patterns of oropharyngeal swallowing in multiple sclerosis. Clin Neurophysiol. 2013;124(8):1638–45.
4. Bergamaschi R, Crivelli P, Rezzani C, Patti F, Solaro C, Rossi P, Restivo D, Maimone D, Romani A, Bastianello S, Tavazzi E, D'Amico E, Montomoli C, Cosi V. The DYMUS questionnaire for the assessment of dysphagia in multiple sclerosis. J Neurol Sci. 2008;269(1–2):49–53.
5. Bergamaschi R, Rezzani C, Minguzzi S, Amato MP, Patti F, Marrosu MG, Bonavita S, Grasso MG, Ghezzi A, Rottoli M, Gasperini C, Restivo D, Maimone D, Rossi P, Stromillo ML, Montomoli C, Solaro C, DYMUS Group. Validation of the DYMUS questionnaire for the assessment of dysphagia in multiple sclerosis. Funct Neurol. 2009;24(3):159–62.
6. Bester M, Lazar M, Petracca M, Babb JS, Herbert J, Grossman RI, Inglese M. Tract-specific white matter correlates of fatigue and cognitive impairment in benign multiple sclerosis. J Neurol Sci. 2013;330(1–2):61–6.
7. Bharucha AE, Dunivan G, Goode PS, Lukacz ES, Markland AD, Matthews CA, Mott L, Rogers RG, Zinsmeister AR, Whitehead WE, Rao SS, Hamilton FA. Epidemiology, pathophysiology, and classification of fecal incontinence: state of the science summary for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop. Am J Gastroenterol. 2015;110(1):127–36.

8. Bharucha AE, Zinsmeister AR, Schleck CD, Melton 3rd LJ. Bowel disturbances are the most important risk factors for late onset fecal incontinence: a population-based case-control study in women. Gastroenterology. 2010;139(5):1559–66.
9. Calcagno P, Ruoppolo G, Grasso MG, De Vincentiis M, Paolucci S. Dysphagia in multiple sclerosis–prevalence and prognostic factors. Acta Neurol Scand. 2002;105:40–3.
10. Chia YW, Gill KP, Jameson JS, Forti AD, Henry MM, Swash M, Shorvon PJ. Paradoxical puborectalis contraction is a feature of constipation in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry. 1996;60(1):31–5.
11. Danesh-Sani SA, Rahimdoost A, Soltani M, Ghiyasi M, Haghdoost N, Sabzali-Zanjankhah S. Clinical assessment of orofacial manifestations in 500 patients with multiple sclerosis. J Oral Maxillofac Surg. 2013;71:290–4.
12. De Pauw A, Dejaeger E, D'hooghe B, Carton H. Dysphagia in multiple sclerosis. Clin Neurol Neurosurg. 2002;104(4):345–51.
13. Drake MJ, Fowler CJ, Griffiths D, Mayer E, Paton JF, Birder L. Neural control of the lower urinary and gastrointestinal tracts: supraspinal CNS mechanisms. Neurourol Urodyn. 2010;29(1):119–27.
14. Faaborg PM, Christensen P, Kvitsau B, Buntzen S, Laurberg S, Krogh K. Long-term outcome and safety of transanal colonic irrigation for neurogenic bowel dysfunction. Spinal Cord. 2009;47(7):545–9.
15. Gill KP, Chia YW, Henry MM, Shorvon PJ. Defecography in multiple sclerosis patients with severe constipation. Radiology. 1994;191(2):553–6.
16. Glick ME, Meshkinpour H, Haldeman S, Bhatia NN, Bradley WE. Colonic dysfunction in multiple sclerosis. Gastroenterology. 1982;83(5):1002–7.
17. Guinet A, Jousse M, Damphousse M, Hubeaux K, Le Breton F, Sheikh Ismael S, Amarenco G. Modulation of the rectoanal inhibitory reflex (RAIR): qualitative and quantitative evaluation in multiple sclerosis. Int J Colorectal Dis. 2011;26(4):507–13.
18. Haldeman S, Glick M, Bhatia NN, Bradley WE, Johnson B. Colonometry, cystometry, and evoked potentials in multiple sclerosis. Arch Neurol. 1982;39:698–701.
19. Hamdy S, Aziz Q, Rothwell JC, Singh KD, Barlow J, Hughes DG, Tallis RC, Thompson DG. The cortical topography of human swallowing musculature in health and disease. Nat Med. 1996;2(11):1217–24.
20. Hamdy S, Aziz Q, Rothwell JC, Power M, Singh KD, Nicholson DA, Tallis RC, Thompson DG. Recovery of swallowing after dysphagic stroke relates to functional reorganization in the intact motor cortex. Gastroenterology. 1998;115(5):1104–12.
21. Herrera W, Zeligman BE, Gruber J, Jones M, Monica C, Pautler R, Wriston R, Cain M, Prescott T, Cobble N, Burks JS. Dysphagia in multiple sclerosis: clinical and videofluoroscopic correlations. J Neurol Rehabil. 1990;4:1–8.
22. Hinds JP, Eidelman BH, Wald A. Prevalence of bowel dysfunction in multiple sclerosis. A population survey. Gastroenterology. 1990;98:1538–42.
23. Jameson JS, Rogers J, Chia YW, Misiewicz JJ, Henry MM, Swash M. Pelvic floor function in multiple sclerosis. Gut. 1994;35:388–90. (PMID: 8150353).
24. Jayasekeran V, Singh S, Tyrrell P, Michou E, Jefferson S, Mistry S, Gamble E, Rothwell J, Thompson D, Hamdy S. Adjunctive functional pharyngeal electrical stimulation reverses swallowing disability after brain lesions. Gastroenterology. 2010;138(5):1737–46.
25. Kuhtz-Buschbeck JP, van der Horst C, Wolff S, Filippow N, Nabavi A, Jansen O, Braun PM. Activation of the supplementary motor area (SMA) during voluntary pelvic floor muscle contractions--an fMRI study. Neuroimage. 2007;35(2):449–57.
26. Kurtzke JF. Rating neurologic impairment in multiple sclerosis. Neurology. 1983;33:1444–52.
27. Levinthal DJ, Rahman A, Nusrat S, O'Leary M, Heyman R, Bielefeldt K. Adding to the burden: gastrointestinal symptoms and syndromes in multiple sclerosis. Mult Scler Int. 2013;2013:319201.

28. Mathers SE, Ingram DA, Swash M. Electrophysiology of motor pathways for sphincter control in multiple sclerosis. J Neurol Neurosurg Psychiatry. 1990;53(11):955–60.
29. McClurg D, Hagen S, Hawkins S, Lowe-Strong A. Abdominal massage for the alleviation of constipation symptoms in people with multiple sclerosis: a randomized controlled feasibility study. Mult Scler. 2011;17(2):223–33.
30. Munteis E, Andreu M, Martinez-Rodriguez J, Ois A, Bory F, Roquer J. Manometric correlations of anorectal dysfunction and biofeedback outcome in patients with multiple sclerosis. Mult Scler. 2008;14(2):237–42.
31. Munteis E, Andreu M, Téllez MJ, Mon D, Ois A, Roquer J. Anorectal dysfunction in multiple sclerosis. Mult Scler. 2006;12(2):215–8.
32. Namey M. Elimination dysfunction in multiple sclerosis. Int J MS Care. 2012;14(S1):1–26.
33. Nigro S, Passamonti L, Riccelli R, Toschi N, Rocca F, Valentino P, Nisticò R, Fera F, Quattrone A. Structural 'connectomic' alterations in the limbic system of multiple sclerosis patients with major depression. Mult Scler. 2015;21(8):1003–12.
34. Nordenbo AM, Andersen JR, Andersen JT. Disturbances of ano-rectal function in multiple sclerosis. J Neurol. 1996;243(6):445–51.
35. Nusrat S, Gulick E, Levinthal D, Bielefeldt K. Anorectal dysfunction in multiple sclerosis: a systematic review. ISRN Neurol. 2012; http://dx.doi.org/10.5402/2012/376023. Article ID 376023.
36. Nusrat S, Levinthal D, Bielefeldt K. Hospitalization rates and discharge status in multiple sclerosis. Mult Scler Int. 2013;2013:436929.
37. Park JW, Oh JC, Lee JW, Yeo JS, Ryu KH. The effect of 5Hz high-frequency rTMS over contralesional pharyngeal motor cortex in post-stroke oropharyngeal dysphagia: a randomized controlled study. Neurogastroenterol Motil. 2013;25(4):324–e250.
38. Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain. 1937;60(4):389–443.
39. Poorjavad M, Derakhshandeh F, Etemadifar M, Soleymani B, Minagar A, Maghzi AH. Oropharyngeal dysphagia in multiple sclerosis. Mult Scler. 2010;16:362–5.
40. Preziosi G, Gosling J, Raeburn A, Storrie J, Panicker J, Emmanuel A. Transanal irrigation for bowel symptoms in patients with multiple sclerosis. Dis Colon Rectum. 2012;55(10):1066–73.
41. Preziosi G, Raptis DA, Raeburn A, Thiruppathy K, Panicker J, Emmanuel A. Gut dysfunction in patients with multiple sclerosis and the role of spinal cord involvement in the disease. Eur J Gastroenterol Hepatol. 2013;25(9):1044–50.
42. Preziosi G, Raptis DA, Storrie J, Raeburn A, Fowler CJ, Emmanuel A. Bowel biofeedback treatment in patients with multiple sclerosis and bowel symptoms. Dis Colon Rectum. 2011;54(9):1114–21.
43. Restivo DA, Casabona A, Centonze D, Marchese-Ragona R, Maimone D, Pavone A. Pharyngeal electrical stimulation for dysphagia associated with multiple sclerosis: a pilot study. Brain Stimul. 2013;6(3):418–23.
44. Restivo DA, Marchese-Ragona R, Patti F, Solaro C, Maimone D, Zappalá G, Pavone A. Botulinum toxin improves dysphagia associated with multiple sclerosis. Eur J Neurol. 2011;18(3):486–90.
45. Schrum A, Wolff S, van der Horst C, Kuhtz-Buschbeck JP. Motor cortical representation of the pelvic floor muscles. J Urol. 2011;186(1):185–90.
46. Sørensen M, Lorentzen M, Petersen J, Christiansen J. Anorectal dysfunction in patients with urologic disturbance due to multiple sclerosis. Dis Colon Rectum. 1991;34(2):136–9.
47. Swash M, Snooks SJ, Chalmers DH. Parity as a factor in incontinence in multiple sclerosis. Arch Neurol. 1987;44(5):504–8.
48. Tassorelli C, Bergamaschi R, Buscone S, Bartolo M, Furnari A, Crivelli P, Alfonsi E, Alberici E, Bertino G, Sandrini G, Nappi G. Dysphagia in multiple sclerosis: from pathogenesis to diagnosis. Neurol Sci. 2008;29:S360–3.

49. Thomas FJ, Wiles CM. Dysphagia and nutritional status in multiple sclerosis. J Neurol. 1999;246:677–82.
50. Turnbull GK, Hamdy S, Aziz Q, Singh KD, Thompson DG. The cortical topography of human anorectal musculature. Gastroenterology. 1999;117:32–9.
51. Waldron DJ, Horgan PG, Patel FR, Maguire R, Given HF. Multiple sclerosis: assessment of colonic and anorectal function in the presence of faecal incontinence. Int J Colorectal Dis. 1993;8(4):220–4.
52. Weber J, Grise P, Roquebert M, Hellot MF, Mihout B, Samson M, Beuret-Blanquart F, Pasquis P, Denis P. Radiopaque markers transit and anorectal manometry in 16 patients with multiple sclerosis and urinary bladder dysfunction. Dis Colon Rectum. 1987;30(2):95–100.
53. Wiesner W, Wetzel SG, Kappos L, Hoshi MM, Witte U, Radue EW, Steinbrich W. Swallowing abnormalities in multiple sclerosis: correlation between videofluoroscopy and subjective symptoms. Eur Radiol. 2002;12(4):789–92.
54. Wiesel PH, Norton C, Roy AJ, Storrie JB, Bowers J, Kamm MA. Gut focused behavioural treatment (biofeedback) for constipation and faecal incontinence in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2000;69(2):240–3.

Chapter 18
The Neurology of Whipple's Disease

Ribal Bassil and Carolina Ionete

Abstract Whipple's disease is a systemic illness caused by an infection with a bacterium of the *Actinomycetes* species called *Tropheryma whipplei*. The infection primarily causes gastroenteritis and malabsorption; however, it could also infect other target organs including the central nervous system (CNS). CNS involvement manifests as a wide spectrum of symptoms such as change in mental status, myoclonus, ophthalmoplegia, and ataxia. Whipple's disease is rare and mostly presents with nonspecific symptoms, therefore requiring a high clinical suspicion for prompt diagnosis. Early initiation of antibiotherapy could prevent bacterial dissemination and produce a complete resolution of symptoms.

Keywords *Tropheryma whipplei* • Whipple's disease • Central nervous system • Oculomasticatory myorhythmia • Oculofacial skeletal myorhythmia

Introduction

In 1907, George Hoyt Whipple, an American physician and Nobel Prize recipient, described a case of a 36-year-old physician who developed malabsorption with diarrhea, weight loss, and arthropathy and subsequently passed away 5 years later of complications of his disease. On autopsy, the identification of intestinal fat and lipid-burdened mononuclear cells prompted Whipple to call the disease "intestinal lipodystrophy."

Years later, further investigation of the disease revealed a systemic illness primarily affecting the gastrointestinal tract as well as other target organs including the heart, lungs, eyes, skin, and central nervous system (CNS). The disease became known as Whipple's disease in 1949 [1].

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Although Whipple's disease is best known for its systemic and gastrointestinal manifestations, neurological involvement is now very well recognized as either a complication of the systemic disorder or as primary presenting symptom.

Epidemiology

Whipple's disease is rare, making incidence and prevalence analyses range widely from one study to the other. It is most likely underdiagnosed given the nonspecific symptoms at the time of presentation and the absence of gastrointestinal involvement in many cases. Nevertheless, the available literature shows that Whipple's disease primarily affects middle-aged Caucasian men with 4:1 men to women ratio. The mean age of onset is around 50–55 years [2].

Etiology

While studying and staining tissues isolated from the gastrointestinal tract of the "intestinal lipodystrophy" case, George Hoyt Whipple interestingly described a "peculiar rod-shaped organism" that may or may not be associated with the etiology of the disease. More than 50 years later, a periodic acid-Schiff (PAS) weakly gram-positive bacillus was identified in the intestinal mucosa macrophages of similar cases [3, 4]. The organism was subsequently named *Tropheryma whippelii* (TW), with the name later changed to *Tropheryma whipplei*, following the successful tissue culture of the organism [5], although the previous nomenclature remains widely in use.

Tropheryma whipplei is ubiquitously expressed in the environment, including in the soil and in sewage water [6]. Furthermore, the organism has been isolated from the saliva and stool of clinically affected patients as well as healthy controls [7–9]. Taken together, this suggests both a genetic predisposition and environmental factors as important players in the bacterium pathogenesis. Although the evidence supporting a genetic predisposition to Whipple's disease is scarce, an association with HLA alleles DRB1*13 and DQB1*06 has been recently described [10].

To date, humans are the only known host for the bacterium. While exposure to the organism may be uneventful in some, in others it may lead to a self-limiting or chronic gastroenteritis that may progress to a chronic carrier or chronic disease state. Many cases with isolated non-gastrointestinal organ involvement have been described; however, it is hard to prove the absence of a preceding remote history of gastroenteritis at the time of the bacterium inoculation.

Given the predominant gastrointestinal manifestation of Whipple's disease and the organism detection in sewage water and stools, it is thought that *Tropheryma whipplei* is transmitted through the fecal-oral route. Although the bacterium has also been detected in human saliva, to date, there is no evidence of transmission via bodily fluids. Little is known regarding the modes of dissemination of the organism

once ingested; however the systemic and multi-organ involvement suggests a hematogenous and lymphatic spread.

Clinical Manifestations

Systemic Manifestations

Multiple organ systems could be affected in Whipple's disease either individually or in combination (Table 18.1). The list includes but is not limited to the gastrointestinal tract, lymphatics, musculoskeletal system, heart, lung, CNS, and, to a lesser extent, the peripheral nervous system (PNS).

A classical Whipple's disease clinical picture is a patient presenting with gastrointestinal and systemic involvement. The main presenting symptoms include diarrhea, abdominal cramping, and weight loss. Systemic symptoms are nonspecific and include fever, lymphadenopathy, and arthralgias. In many cases and in retrospect, arthralgias preceded the other manifestations and diagnosis by years [11–13]. If the disease remains untreated, chronic complications of malabsorption become

Table 18.1 Common clinical manifestations of Whipple's disease

Gastrointestinal
Diarrhea
Steatorrhea
Abdominal pain
Bloating
Malabsorption
Weight loss
Systemic
Fever
Lymphadenopathy
Arthropathy
Cardiac
Endocarditis
Pericarditis
Congestive heart failure
Pulmonary
Pleural effusion
Chest pain
Ocular
Uveitis
Keratitis
Retinitis
Papilledema

evident. Most noteworthy is vitamin D deficiency leading to osteomalacia and hyperpigmentation and vitamin B12 deficiency leading to anemia [14, 15].

Cardiac involvement is a well-recognized complication of Whipple's disease. In fact, in the right clinical setting, *Tropheryma whipplei* should be considered in the differential diagnosis of endocarditis, pericarditis, or congestive heart failure with initial negative workup [16–18].

Pulmonary manifestations could range from asymptomatic lymphadenopathy to dyspnea, chest pain, or pleural effusion [19].

Ocular involvement is not uncommon and mainly results in uveitis, although keratitis, retinitis, and optic neuritis have been described [20, 21].

Central Nervous System Manifestations

CNS infections result in small, sometimes confluent granulomas with preferential involvement of the cerebral cortical and deep gray matter. The granulomas consist of a PAS-positive macrophage core embedded within a large reactive astrocytic surface [22–24] (Fig. 18.1). It remains to be determined whether direct *Tropheryma*

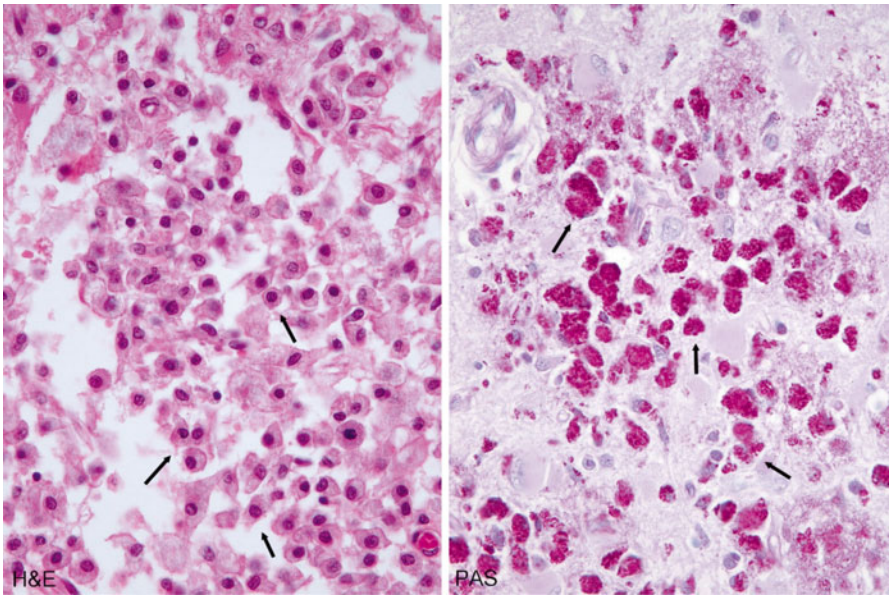

Fig. 18.1 A labeled composite image of the 40x images of CNS Whipple's disease. Autopsy specimen of the hippocampus from a 25-year-old male with a 1-year history of progressive dementia, supranuclear ophthalmoplegia, and right arm myoclonus. The H&E stain (*left image*) shows a large cluster of foamy macrophages (*arrows*) in the gray matter. The PAS stain (*right image*) shows PAS+ cytoplasmic inclusions consistent with *T. whipplei* bacteria within the macrophages (*arrows*). Original magnification, both images, 40× (Reproduced with permission from Dr. T Smith)

Table 18.2 Common CNS manifestations of Whipple's disease

Cerebral atrophy
Headache
Cognitive decline
Psychiatric signs
Myoclonus
Supranuclear gaze palsy
Autonomic dysfunction
Hypersomnia
Hyperphasia
OMM
OFSM
Aphasia
Dysarthria
Motor weakness
Paresis
Seizure
Ataxia
Nystagmus
Optic neuritis
Cranial nerve palsy

whipplei pathogenesis or the associated inflammatory granulomatous reaction, or both are responsible for the CNS pathology.

Neurological involvement may cause a wide spectrum of nonspecific signs and symptoms such as brain atrophy or headaches [13]. Some manifestations are more easily anatomically localizable depending on the underlying involved structures (Table 18.2).

Cognitive change is the most common neurological presentation and includes memory difficulty and behavioral changes. Nonspecific psychiatric manifestations are also common especially in the setting of cognitive decline [13, 25].

Vision could be compromised by direct ocular, optic nerve, or optic chiasm involvement. Furthermore, eye movement disorder should raise a high degree of suspicion for Whipple's disease, as it is the second most common presenting symptom. Ophthalmoplegia usually signals brainstem or cranial nerve involvement with supranuclear gaze palsy or vertical ophthalmoparesis [26–30].

Oculomasticatory myorhythmia (OMM) and oculofacial skeletal myorhythmia (OFSM) are rare ocular movement disorders that have not been associated with any pathology other than Whipple's disease [31, 32]. Although it is an uncommon presentation, it is considered pathognomonic of the disease. OMM consists of constant synchronous ocular pendular vergence oscillations with concurrent contractions of the masticatory muscles [33]. OFSM is similar to OMM in addition to synchronous rhythmic movements of the extremities and persists during sleep [34].

Focal cerebral involvement could result in symptoms such as dysarthria, aphasia, weakness, or paresis corresponding to the localization of the lesions [35]. Ataxia and nystagmus point to cerebellar involvement [36]. Cranial nerve palsies have also been reported [13].

Movement disorders include myoclonus or rarely Parkinsonism [34, 37, 38]. Seizures are most likely secondary to focal cortical lesions or limbic involvement [27, 39]. Autonomic dysfunction, hypersomnia, and hyperphagia signal hypothalamic involvement [34, 35, 40]. Large or confluent granulomas could present as space-occupying lesions exerting mass effect [41]. If obstructing the CSF circulation, hydrocephalus could be seen [42].

Myelopathy, either as isolated presentation or in concurrence with other CNS symptoms, has been described [30, 43, 44]. While Whipple's disease of the CNS is well established, PNS involvement is less common.

Diagnosis

Tropheryma whipplei has proven very difficult to culture [45]. Tissue biopsy and staining are impractical, especially in the setting of Whipple's disease with no gastrointestinal manifestation.

The diagnostic tool of choice is the isolation of a single bacterial 16S ribosomal RNA gene sequence by polymerase chain reaction (PCR) technique. In fact, it is the analysis of the bacterial gene by PCR that allowed the classification of the bacterium as novel *Actinomycetes* [46].

Saliva and stool sample PCR is not a reliable diagnostic study as it had been found to be positive in healthy individuals, presumed asymptomatic carriers [7, 8]. Therefore, the identification of the bacterium by PCR in the target organ is critical. Luckily, in the setting of CNS involvement, a tissue biopsy is rarely indicated, as CSF PCR is the cornerstone for diagnosis [44]. Of note, CSF fluid analysis could be unremarkable or it could demonstrate mildly elevated protein level or white blood cell count [13, 47].

Electroencephalography is non-diagnostic and usually shows generalized slowing or nonspecific findings corresponding to potential focal lesions [27].

Brain imaging studies such as CT scan or MRI are also nonspecific (Figs. 18.2 and 18.3). The findings range from normal brain to diffuse atrophy [13]. Lesions range from focal to scattered, contrast-enhancing or non-enhancing, and sometimes ring-enhancing lesion [30, 41]. Cases with space-occupying lesions complicated by hydrocephalus have been described [42, 48]. Spinal cord involvement has been reported; therefore, imaging would be indicated if the clinical presentation is suggestive of it.

Treatment

An infectious etiology of Whipple's disease has been proposed long before *Tropheryma whipplei* was identified; therefore, there is a well-documented history of successful antibiotherapy [49]. However, the emergence of many resistant or relapsing cases or subsequent presentations with neurological symptoms mandated a choice of antibiotics with excellent CNS penetration and good patients' tolerance [50].

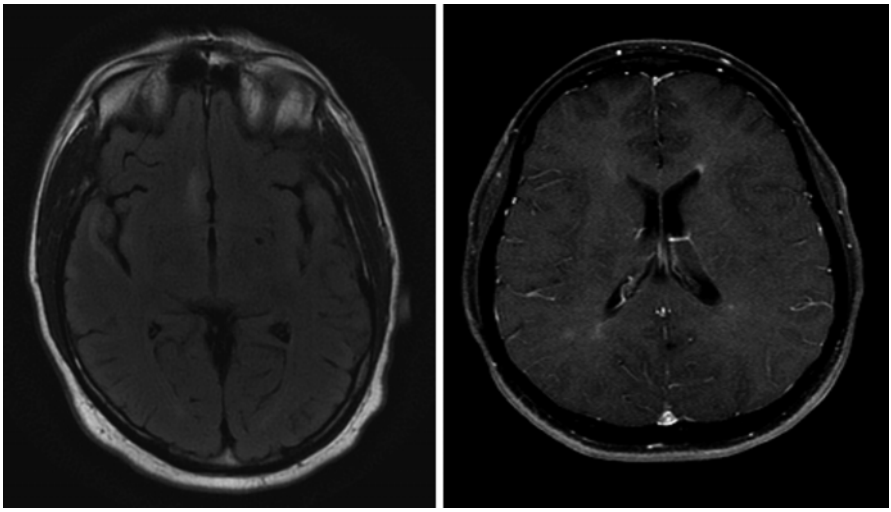

Fig. 18.2 Axial noncontrast FLAIR and axial T1 with gadolinium images demonstrate enhancing abnormally increased T2 signal intensity in the bilateral temporal lobe and right medial frontal lobe in a 45-year-old man presented with subacute rapid progressive dementia and biopsy proven Whipple's disease

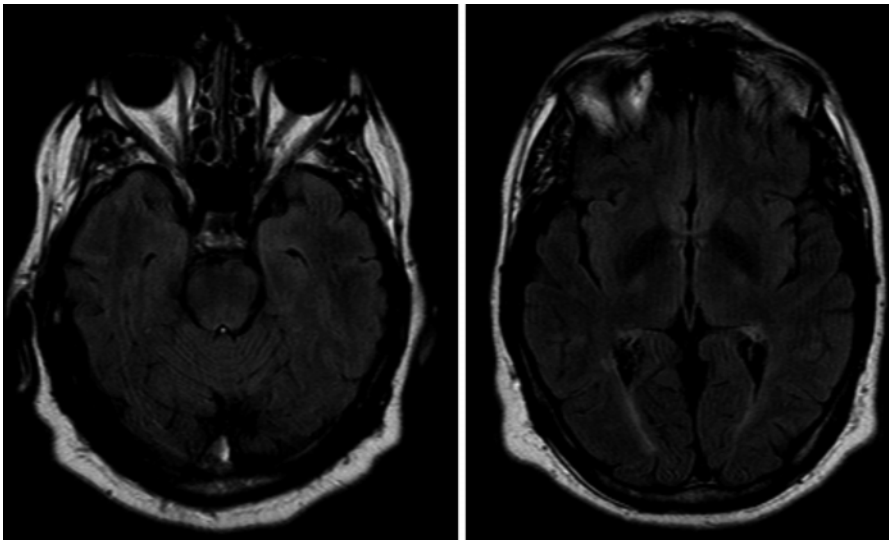

Fig. 18.3 Axial noncontrast FLAIR demonstrates abnormally increased T2 signal intensity in the bilateral medial temporal poles, pyramidal tracts (anterior aspect of the midbrain and internal capsule), and posterior midbrain involving periaqueductal region (quadrigeminal plate) in a 37-year-old woman, diagnosed by brain biopsy with Whipple's disease after she presented with oculomasticatory myokymia, vertical gaze palsy, and delirium

The treatment duration is not well defined; however, most studies show a preferable outcome with 2–4 weeks of intravenous agent administration, followed by 1 year of oral therapy. For patients with endocarditis or CNS infection, a longer 4-week course of intravenous antibiotherapy is recommended [51].

With regard to the choice of antibiotics and length of treatment, current guidelines recommend the administration of intravenous ceftriaxone at 2 g once daily for 2–4 weeks followed by oral trimethoprim-sulfamethoxazole (TMP-SMX) double-strength tablet twice daily for 1–2 years [52, 53]. Of note, several authors recommend ceftriaxone at 2 g twice a day during the parenteral treatment phase.

It would be prudent to have all patients receiving TMP-SMX on daily folic acid supplementation as TMP, a dihydrofolate reductase inhibitor, may cause folate deficiency.

For patients with penicillin or ceftriaxone allergies, the intravenous regimen is substituted with oral TMP-SMX double-strength tablet three times daily plus streptomycin at 1 g intramuscular daily for 2–4 weeks. For cases of sulfa drug allergy, oral TMP-SMX is substituted by oral doxycycline concurrently with hydroxychloroquine. Alternatively, oral cefixime has been used [51].

Prognosis

The use of antibiotics with excellent CNS penetration, both during the acute and chronic maintenance phases, has certainly improved outcomes and has decreased the recurrence rate. Overall, prognosis is good, especially in the absence of significant underlying target organ structural lesions. Clinical improvement is expected within weeks of initiation of therapy, and the success of therapy is judged based on clinical improvement.

Nevertheless, some cases of recurrence while on antibiotics or after completion of chronic therapy have been described. It is unclear whether this is related to host factors such as poor compliance or immune suppression or whether it is due to a change in the bacterial pathogenic or resistance profile. Therefore, in case of recurrence or failure of therapy, it is recommended that treatment should be reinstituted or the antibiotic regimen changed.

Although routine tissue or CSF PCR for *Tropheryma whipplei* has been considered, its value remains uncertain. However, in cases of recurrence or initial therapy failure, it would be reasonable to analyze the CSF or target tissue when feasible by PCR after completion of the antibiotic course and to determine the best course of action accordingly [54]. In fact, some authors advocate for lifetime prophylactic treatment following initial treatment failure [55].

Immune reconstitution inflammatory syndrome (IRIS) is the main complication following the initiation of treatment in Whipple's disease. This consists of a severe inflammatory process resulting in high-grade fever or other systemic symptoms. The population at risk includes patients previously on immunosuppressive treatment

or patients with Whipple's disease of the CNS [56]. If the reaction is severe enough, administration of corticosteroid therapy is indicated.

Conclusion

Timely diagnosis of Whipple's disease is challenging, as the presenting symptoms are highly variable and could virtually involve any organ system. Therefore, medical professionals of all specialties should be familiar with this diagnosis and keep a high clinical suspicion especially in the setting of atypical cases with negative initial workup. The prompt initiation of antibiotherapy has changed the natural course of this chronic, potentially life-threatening disease. However, routine follow-up is warranted as failure of therapy or disease recurrence has been well documented.

References

1. Black-Schaffer B. The tinctorial demonstration of a glycoprotein in Whipple's disease. Proc Soc Exp Biol Med. 1949;72(1):225–7.
2. Moos V, Schneider T. Changing paradigms in Whipple's disease and infection with Tropheryma whipplei. Eur J Clin Microbiol Infect Dis. 2011;30(10):1151–8.
3. Chears Jr WC, Ashworth CT. Electron microscopic study of the intestinal mucosa in Whipple's disease. Demonstration of encapsulated bacilliform bodies in the lesion. Gastroenterology. 1961;41:129–38.
4. Yardley JH, Hendrix TR. Combined electron and light microscopy in Whipple's disease. Demonstration of "bacillary bodies" in the intestine. Bull Johns Hopkins Hosp. 1961;109:80–98.
5. Raoult D, Birg ML, La Scola B, Fournier PE, Enea M, Lepidi H, et al. Cultivation of the bacillus of Whipple's disease. N Engl J Med. 2000;342(9):620–5.
6. Maiwald M, Schuhmacher F, Ditton HJ, von Herbay A. Environmental occurrence of the Whipple's disease bacterium (Tropheryma whippelii). Appl Environ Microbiol. 1998;64(2):760–2.
7. Fenollar F, Trani M, Davoust B, Salle B, Birg ML, Rolain JM, et al. Prevalence of asymptomatic Tropheryma whipplei carriage among humans and nonhuman primates. J Infect Dis. 2008;197(6):880–7.
8. Street S, Donoghue HD, Neild GH. Tropheryma whippelii DNA in saliva of healthy people. Lancet. 1999;354(9185):1178–9.
9. Zinkernagel AS, Gmur R, Fenner L, Schaffner A, Schoedon G, Schneemann M. Marginal and subgingival plaque – a natural habitat of Tropheryma whipplei? Infection. 2003;31(2):86–91.
10. Martinetti M, Biagi F, Badulli C, Feurle GE, Muller C, Moos V, et al. The HLA alleles DRB1*13 and DQB1*06 are associated to Whipple's disease. Gastroenterology. 2009;136(7):2289–94.
11. Durand DV, Lecomte C, Cathebras P, Rousset H, Godeau P. Whipple disease. Clinical review of 52 cases. The SNFMI Research Group on Whipple Disease. Societe Nationale Francaise de Medecine Interne. Medicine. 1997;76(3):170–84.
12. Lagier JC, Lepidi H, Raoult D, Fenollar F. Systemic Tropheryma whipplei: clinical presentation of 142 patients with infections diagnosed or confirmed in a reference center. Medicine. 2010;89(5):337–45.

13. Louis ED, Lynch T, Kaufmann P, Fahn S, Odel J. Diagnostic guidelines in central nervous system Whipple's disease. Ann Neurol. 1996;40(4):561–8.
14. Di Stefano M, Jorizzo RA, Brusco G, Cecchetti L, Sciarra G, Loperfido S, et al. Bone mass and metabolism in Whipple's disease: the role of hypogonadism. Scand J Gastroenterol. 1998;33(11):1180–5.
15. Mori K, Ando I, Kukita A. Generalized hyperpigmentation of the skin due to vitamin B12 deficiency. J Dermatol. 2001;28(5):282–5.
16. Geissdorfer W, Moos V, Moter A, Loddenkemper C, Jansen A, Tandler R, et al. High frequency of Tropheryma whipplei in culture-negative endocarditis. J Clin Microbiol. 2012;50(2):216–22.
17. Lepidi H, Fenollar F, Dumler JS, Gauduchon V, Chalabreysse L, Bammert A, et al. Cardiac valves in patients with Whipple endocarditis: microbiological, molecular, quantitative histologic, and immunohistochemical studies of 5 patients. J Infect Dis. 2004;190(5):935–45.
18. Wendler D, Mendoza E, Schleiffer T, Zander M, Maier M. Tropheryma whippelii endocarditis confirmed by polymerase chain reaction. Eur Heart J. 1995;16(3):424–5.
19. Winberg CD, Rose ME, Rappaport H. Whipple's disease of the lung. Am J Med. 1978;65(5):873–80.
20. Font RL, Rao NA, Issarescu S, McEntee WJ. Ocular involvement in Whipple's disease: light and electron microscopic observations. Arch Ophthalmol. 1978;96(8):1431–6.
21. Rickman LS, Freeman WR, Green WR, Feldman ST, Sullivan J, Russack V, et al. Brief report: uveitis caused by Tropheryma whippelii (Whipple's bacillus). N Engl J Med. 1995;332(6):363–6.
22. Knox DL, Green WR, Troncoso JC, Yardley JH, Hsu J, Zee DS. Cerebral ocular Whipple's disease: a 62-year odyssey from death to diagnosis. Neurology. 1995;45(4):617–25.
23. Romanul FC, Radvany J, Rosales RK. Whipple's disease confined to the brain: a case studied clinically and pathologically. J Neurol Neurosurg Psychiatry. 1977;40(9):901–9.
24. Schochet Jr SS, Lampert PW. Granulomatous encephalitis in Whipple's disease. Electron miscroscopic observations. Acta Neuropathol. 1969;13(1):1–11.
25. Manzel K, Tranel D, Cooper G. Cognitive and behavioral abnormalities in a case of central nervous system Whipple disease. Arch Neurol. 2000;57(3):399–403.
26. Averbuch-Heller L, Paulson GW, Daroff RB, Leigh RJ. Whipple's disease mimicking progressive supranuclear palsy: the diagnostic value of eye movement recording. J Neurol Neurosurg Psychiatry. 1999;66(4):532–5.
27. Finelli PF, McEntee WJ, Lessell S, Morgan TF, Copetto J. Whipple's disease with predominantly neuroophthalmic manifestations. Ann Neurol. 1977;1(3):247–52.
28. Lee AG. Whipple disease with supranuclear ophthalmoplegia diagnosed by polymerase chain reaction of cerebrospinal fluid. J Neuroophthalmol. 2002;22(1):18–21.
29. Rajput AH, McHattie JD. Ophthalmoplegia and leg myorhythmia in Whipple's disease: report of a case. Mov Disord. 1997;12(1):111–4.
30. Kremer S, Besson G, Bonaz B, Pasquier B, Le Bas JF, Grand S. Diffuse lesions in the CNS revealed by MR imaging in a case of Whipple disease. AJNR Am J Neuroradiol. 2001;22(3):493–5.
31. Hausser-Hauw C, Roullet E, Robert R, Marteau R. Oculo-facio-skeletal myorhythmia as a cerebral complication of systemic Whipple's disease. Mov Disord. 1988;3(2):179–84.
32. Schwartz MA, Selhorst JB, Ochs AL, Beck RW, Campbell WW, Harris JK, et al. Oculomasticatory myorhythmia: a unique movement disorder occurring in Whipple's disease. Ann Neurol. 1986;20(6):677–83.
33. Revilla FJ, de la Cruz R, Khardori N, Espay AJ. Teaching NeuroImage: oculomasticatory myorhythmia: pathognomonic phenomenology of Whipple disease. Neurology. 2008;70(6), e25.
34. Xia C, Duquette A, Frucht S, Lafontaine AL. Whipple's disease presenting with segmental myoclonus and hypersomnia. Mov Disord. 2012;27(10):1216–7.

35. Bleibel JM, Cabane J, Kardouss J, Wechsler B, Poisson M, Buge A. Hypothalamic form of Whipple's disease. Favorable effect of rifampicin. Ann Med Interne. 1983;134(8):723–7.
36. Matthews BR, Jones LK, Saad DA, Aksamit AJ, Josephs KA. Cerebellar ataxia and central nervous system whipple disease. Arch Neurol. 2005;62(4):618–20.
37. Stoupel N, Monseu G, Pardoe A, Heimann R, Martin JJ. Encephalitis with myoclonus in Whipple's disease. J Neurol Neurosurg Psychiatry. 1969;32(4):338–43.
38. Stamboulis E, Kararizou E, Manta P, Grivas I. Segmental myoclonus in Whipple's disease. Electromyogr Clin Neurophysiol. 1995;35(2):113–6.
39. Mohm J, Naumann R, Schuler U, Ehninger G. Abdominal lymphomas, convulsive seizure and coma: a case of successfully treated, advanced Whipple's disease with cerebral involvement. Eur J Gastroenterol Hepatol. 1998;10(10):893–5.
40. Voderholzer U, Riemann D, Gann H, Hornyak M, Juengling F, Schumacher M, et al. Transient total sleep loss in cerebral Whipple's disease: a longitudinal study. J Sleep Res. 2002;11(4):321–9.
41. Wroe SJ, Pires M, Harding B, Youl BD, Shorvon S. Whipple's disease confined to the CNS presenting with multiple intracerebral mass lesions. J Neurol Neurosurg Psychiatry. 1991;54(11):989–92.
42. Seneca V, Imperato A, Colella G, Cioffi V, Mariniello G, Gangemi M. Recurrent acute obstructive hydrocephalus as clinical onset of cerebral Whipple's disease. Clin Neurol Neurosurg. 2010;112(8):717–21.
43. Clarke CE, Falope ZF, Abdelhadi HA, Franks AJ. Cervical myelopathy caused by Whipple's disease. Neurology. 1998;50(5):1505–6.
44. Messori A, Di Bella P, Polonara G, Logullo F, Pauri P, Haghighipour R, et al. An unusual spinal presentation of Whipple disease. AJNR Am J Neuroradiol. 2001;22(5):1004–8.
45. Schoedon G, Goldenberger D, Forrer R, Gunz A, Dutly F, Hochli M, et al. Deactivation of macrophages with interleukin-4 is the key to the isolation of Tropheryma whippelii. J Infect Dis. 1997;176(3):672–7.
46. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med. 1992;327(5):293–301.
47. Halperin JJ, Landis DM, Kleinman GM. Whipple disease of the nervous system. Neurology. 1982;32(6):612–7.
48. Lapointe LR, Lamarche J, Salloum A, Beaudry R. Meningo-ependymitis in Whipple's disease. Can J Neurol Sci. 1980;7(2):163–7.
49. Paulley JW. A case of Whipple's disease (intestinal lipodystrophy). Gastroenterology. 1952;22(1):128–33.
50. Keinath RD, Merrell DE, Vlietstra R, Dobbins 3rd WO. Antibiotic treatment and relapse in Whipple's disease. Long-term follow-up of 88 patients. Gastroenterology. 1985;88(6):1867–73.
51. Schnider PJ, Reisinger EC, Berger T, Krejs GJ, Auff E. Treatment guidelines in central nervous system Whipple's disease. Ann Neurol. 1997;41(4):561–2.
52. Feurle GE, Junga NS, Marth T. Efficacy of ceftriaxone or meropenem as initial therapies in Whipple's disease. Gastroenterology. 2010;138(2):478–86; quiz 11–2.
53. Viteri AL, Greene Jr JF, Chandler Jr JB. Whipple's disease, successful response to sulfamethoxazole-trimethoprim. Am J Gastroenterol. 1981;75(4):309–10.
54. von Herbay A, Ditton HJ, Schuhmacher F, Maiwald M. Whipple's disease: staging and monitoring by cytology and polymerase chain reaction analysis of cerebrospinal fluid. Gastroenterology. 1997;113(2):434–41.
55. Lagier JC, Fenollar F, Lepidi H, Raoult D. Evidence of lifetime susceptibility to Tropheryma whipplei in patients with Whipple's disease. J Antimicrob Chemother. 2011;66(5):1188–9.
56. Feurle GE, Moos V, Schinnerling K, Geelhaar A, Allers K, Biagi F, et al. The immune reconstitution inflammatory syndrome in whipple disease: a cohort study. Ann Intern Med. 2010;153(11):710–7.