Chapter 1
The Immunology of the Gastrointestinal System

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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].

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