Chapter

The Influence of Microbial Metabolites in the Gastrointestinal Microenvironment on Anticancer Immunity

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Abstract

The gastrointestinal (GI) tumour microenvironment is characterised by its unique colonisation with bacteria that are estimated to match the total number of cells in our body. It is becoming increasingly clear that the microbiome and its metabolites are important orchestrators of local and systemic immune responses, anticancer immunity and the host response to cancer therapy. Apart from their role as an energy source, metabolites have been shown to modulate inflammation, immune cell function and cancer cell survival. The polarisation of immune cell subsets by microbial metabolites towards either pro- or antitumorigenic functions strongly affects cancer progression and outcomes. In this chapter, we will discuss the link between microbial metabolites in the GI tumour microenvironment, anticancer immune responses and cancer progression.

Keywords: gastrointestinal tumour environment, host immune response, innate immunity, microbial metabolites, metabolism

1. Introduction

The GI tract is a complex ecosystem, populated by a large variety of bacteria, fungi and viruses that together form the intestinal microbiome. A surprising amount of local and systemic bodily functions are affected by the composition of the microbiome and its produced metabolites. This includes the generation of energy, metabolism of dietary components and synthesis of vitamins as well as regulation of immune responses, behaviour and mood. Perturbations of microbial populations, commonly referred to as dysbiosis, have been associated with a large number of diseases, such as inflammatory bowel disease [1], diabetes [2], obesity [3], autism [4], depression [5] and colorectal cancer [6, 7]. Understanding the reciprocal relationship between the microbiota and immunity has received great attention as it is becoming increasingly clear that inflammatory processes underlie many pathologies. The complexity of microbiome-immune interactions is staggering as not only the presence or absence of bacterial species shape immunity, but metabolites produced and modified by bacteria have a direct effect on the immune system's ability to react to infectious and non-communicable diseases [8].

Microbial metabolites, as the sum of products modified and synthesised by microbiota, can be a useful tool to understand microbiota-driven immune modulation when analysis of bacterial lineages proves difficult. Diversity and abundance of microbial communities varies greatly amongst healthy individuals, whereas metabolic pathways are conserved and stable [9, 10]. Therefore, assessing changes of metabolic pathways and how they affect immunity may provide crucial insights into the role of the GI microenvironment in health and disease. Microbial metabolites are commonly divided into three categories, (1) metabolites produced by bacteria, derived from host products; (2) metabolites modified by bacteria, derived from host products; and (3) metabolites synthesised by bacteria directly.

In the following sections, we will briefly describe GI cancers and components of the GI tract that shape the tumour microenvironment. Furthermore, we will discuss the evidence for connecting changes in the microbiome and its metabolites with carcinogenesis and the role of bacterial metabolites in shaping immunity and in particular anticancer immunity.

2. The GI cancer microenvironment

2.1 The gastrointestinal tract

The gastrointestinal tract starts at the mouth, extends to the anus and includes the oesophagus, stomach, small intestine, large intestine, liver and pancreas. Its main functions are primarily the disruption and digestion of food, absorption of nutrients and elimination of waste products. With the diverse functions of the GI tract, it is not unsurprising that it has a number of diverse environments which are contributed to by various types of immune cells and the multiple bacteria that reside in the GI tract.

Movement of food down the GI tract is facilitated by muscular contractions. Much of the tube that makes up the GI tract is muscle lined to enable this to occur, with sphincters at particular junctures to enable control of food passage. The muscle layers are coated by a mucous membrane which varies depending on the function of that section of the GI tract.

The epithelium that lines the GI tract can be broadly divided into three subtypes, primarily based on their function. Squamous epithelium is found at the start (mouth and oesophagus) and end (anus) of the GI tract providing a protective covering. Secretory epithelium is found in the stomach. Absorptive epithelium is found in both the small and large intestines. The small intestine has numerous fingerlike projections, called villi, that increase the surface area to facilitate absorption of nutrients with interspersed crypts, or glands, which contain the stem cells that give rise to the epithelial cells. The absorptive epithelium of the large intestine is more closely packed with glands specialised for water absorption and mucus-secreting cells to lubricate the passage of faecal material down the GI tract.

The tube that forms the GI tract has a number of layers that lie between the outer muscular wall (the muscularis propria) and the innermost epithelium. The epithelium forms the innermost layer of the mucosa, which has two additional components, the lamina propria (composed of supportive connective tissue) and a thin layer of smooth muscle, the muscularis mucosae. Underneath the mucosa is the submucosa, which contains connective tissue, nerves and lymphatic and blood vessels. The submucosa is surrounded by the outer muscularis propria, the muscle layer whose contractions facilitate passage of material down the GI tract. The supporting tissue surrounding the GI tract is called the adventitia or serosa and contains major nerves and blood vessels.

The GI tract includes two large glands, the liver and pancreas. Both develop from the primitive foregut embryonically and have functions that contribute to digestion by generating digestive fluids. The liver produces bile, which can be stored and concentrated in the gallbladder. When lipids enter the duodenum, neuroendocrine cells of the duodenal mucosa are stimulated to release cholecystokinin-pancreozymin (CCK) causing contraction of the gallbladder releasing bile into the duodenum. Bile acids are emulsifying agents which aid in lipid digestion. Pancreatic secretions reach the duodenum via the pancreatic duct and contain a high content of alkaline bicarbonate ions which assist in neutralising the acidic fluid that has come from the stomach. The pancreas also produces a number of enzymes including trypsin, chymotrypsin, amylase, lipase and carboxypeptidases which are involved in the breakdown of proteins, carbohydrates and lipids.

2.2 Microbiota in the GI tract

Our lifestyle, including diet, exercise, childhood microbial exposure and the use of antibiotics strongly, influences the composition of our microbiota [9, 11–14]. Two phyla of bacteria dominate the human gut *Bacteroidetes* and *Firmicutes*. Over decades the ability to classify bacteria into their genus and species has evolved with technology resulting in numerous reclassifications. Bacteria can be additionally classified into subspecies on the basis of small but relevant differences within a species. Further classifications into strains or serovars, indicating variable immune antigens present on their surface, can be allocated outside nomenclature rules. This level of complexity demonstrates the purpose of studying microbial metabolites in the context of gut immunity, thereby avoiding the complexities of bacterial species, focusing instead on their metabolic output.

Epidemiological data initially made links between bacteria and cancer development. However, identifying the role of bacteria in cancer development has been challenging due to the importance of host factors in cancer susceptibility combined with the ubiquitous nature of bacteria and the prolonged period between introduction of a bacterium and development of overt cancer [15]. This is further complicated by environmental factors which are thought to play a much larger role than genetic makeup in determining the makeup of an individual's microbiota [16].

While the knowledge of outcomes from bacterial interactions with human cells is growing, there is enormous potential for further discovery when accommodating other microbes that populate different levels of the gastrointestinal tract such as fungi and viruses [17].

2.3 Gastrointestinal cancers

Gastrointestinal (GI) cancers are as diverse as the environments of the GI tract and the various cell types found in the GI tract. Squamous cell carcinomas arise in the squamous epithelium of the oral cavity, oesophagus and anus. Those that arise in the oral cavity are considered head and neck cancers rather than GI cancers. Adenocarcinomas are cancers that arise from glandular epithelium and can arise in the oesophagus, stomach, small intestine, pancreas and large intestine. Other cancers that can arise from the GI tract include cholangiocarcinoma, with origin from bile duct cells; hepatocellular carcinoma (HCC), originating from hepatocytes (liver cells); gastrointestinal stromal tumours, originating from the interstitial cells of Cajal which have a role in the control of peristaltic contractions [18]; and neuroendocrine cancers which can arise from neuroendocrine cells throughout the GI tract. Multiple studies examining these GI cancers have demonstrated diverse molecular alterations within cancers that arise from the same cell type in the same

organ of the GI tract, highlighting the multitude of malignancies that can arise in the GI tract [19–27].

Chronic inflammation and infection are intimately associated with the development of cancer, with 15% of global cancer cases in 2012 being attributed to a carcinogenic infection [28]. Examples from the GI tract include HCC with hepatitis B and C virus infections contributing to more than 70% of global HCC diagnoses in 2012 [28]. In gastric adenocarcinoma four molecular subtypes were described by The Cancer Genome Atlas (TCGA) in 2014, one is characterised by Epstein-Barr virus positivity and shows extreme DNA hypermethylation [29]. *Helicobacter pylori* (*H. pylori*) is considered a class I carcinogen by the World Health Organization due to the association of chronic infection with the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma, a form of B cell lymphoma in the stomach [30, 31]. The risk of developing gastric cancer with *H. pylori* is dependent on the virulence factors of the strain causing infection, other environmental factors and host genetics [32–34].

In colorectal cancer studies, *Fusobacterium* subspecies were consistently identified as being differentially present in tumour samples; however, these findings are still limited by small sample sizes [35]. A study of the microbiome in a hereditary form of CRC has implicated oncotoxins produced by co-colonisation with *Bacteroides fragilis* (*B. fragilis*) and *Escherichia coli* (*E. coli*) subspecies in mucosal biofilms. Further animal studies have revealed the bacterial synergy involved in carcinogenesis whereby the *B. fragilis* toxin increases expression of the pro-inflammatory cytokine interleukin (IL)-17 enabling the oncotoxin-producing *E. coli* to invade the mucosa inducing DNA damage in epithelial cells [36].

In addition to bacteria and viruses being implicated in carcinogenesis, parasitic infections have also been implicated in cancer development in the GI tract, with liver fluke infection, particularly *Opisthorchis viverrini,* being associated with the development of cholangiocarcinoma [37].

2.4 Inflammation in the GI tract

Acute inflammation is an integral part of the host defence against pathogens and tissue damage and is also required for the initiation of beneficial antitumour immunity [38, 39]. In contrast, it is ongoing 'smouldering' inflammation that contributes to tumour development, progression, invasion and metastasis [40]. Low-grade inflammation affects the function of immune cells and promotes an immune-suppressive, tumour-promoting phenotype [41, 42]. This in turn is associated with reduced immune surveillance and clearance of tumour cells by the immune system.

Chronic inflammation can be induced through a variety of mechanisms, including chronic infections [43], autoimmunity [44], metabolic disorders [45] and altered microbiota [46, 47]. In the GI tract in particular, the host immune system has to maintain a delicate balance, and pathogens and malignant cells need to be cleared, whereas normal flora has to be tolerated. Disruption of immune tolerance or dysbiosis may result in loss of epithelial barrier function and overstimulation of immune cells, leading to tissue damage and chronic inflammation.

Conditions associated with recurrent or chronic inflammation, such as inflammatory bowel disease (IBD), have been shown to contribute to the risk of developing small and large intestine cancers [48, 49]. Mechanistically, this has been related to increased stimulation of inflammation-promoting immune cells by altered microbiota [46]. As a result, pro-inflammatory cytokines and chemokines are secreted, attract further immune cells into the tissue and polarise them towards tumour-promoting functions [50–52]. Particularly the presence of pathogenic

T-cell subsets, induced by pro-inflammatory cytokines, has been shown to be a predictor of poor prognosis in colorectal cancer patients [53]. Chronic inflammation also contributes to the expansion of oncogenic bacteria thereby re-enforcing disease progression [54]. See **Box 1** for an overview of the immune cell populations involved in intestinal antitumour immune responses.

Dendritic cells
DCs are innate immune cells that develop from myeloid precursors in the bone marrow. They are capable of detecting pathogens or tissue disturbances and initiate an inflammatory response. In the tumour microenvironment, they are thought to engulf dead tumour cells and debris and present fragments thereof to T cells, thus initiating anticancer immune responses.

Macrophages
Macrophages are antigen-presenting cells that arise from either circulating monocytes or embryonic progenitors that persist into adulthood, both giving rise to tissue-specific macrophage populations that are capable of self-renewal [186, 187]. Macrophages serve important functions in immunity, cancer, metabolism and tissue repair. Macrophages play an important role in in the antitumour immune response but can also adopt a pro-tumour phenotype in the tumour environment [188, 189].

Cytotoxic T cells (CD8+ T cells)
CTLs express the CD8 receptor and recognise antigens presented on the surface of antigen-presenting cells. Once primed by this encounter, CTLs are capable of recognising the same antigens and kill target cells expressing the antigen. Tumour-specific CTL responses are crucial for controlling tumour growth.

Helper T cells (CD4+ T cells)
Th cells express the CD4 receptor and support functions of innate and adaptive immune cells by secreting cytokines. Depending on the environment they encounter, Th cells develop into subsets with a wide range of functions [190].
Cytokines, such as IL-12, promote the development of Th1 cells, which are efficient at secreting IFN-γ and TNF-α, important cytokines for antitumour immunity. When naïve $CD4^+$ T cells reside an environment high in IL-6 and TGF-β, they develop into Th17 cells, which promote autoimmunity and are a negative prognostic marker for colorectal cancer [49]. Th cells can develop into immune-suppressive Tregs in the presence of TGF-β when expressing the transcription factor *FOXP3*.

Mucosa-activated invariant T cells (MAIT cells)
MAIT cells reside at mucosal surfaces in the lung and the intestine [163] and are widely distributed in tissue and the systemic blood circulation [164, 165]. They have innate immune cell features but also express a semi-invariant T-cell receptor, which can recognise antigens presented on a monomorphic MHC class 1-related protein (MR1) expressed by antigen-presenting cells [166]. MAIT cells are first responders to a variety of infections caused by bacteria, fungi and viruses through detection of microbial B vitamin antigens.

Innate lymphoid cells (ILCs)
ILCs stem from the lymphoid lineage but have innate immune cell characteristics [109]. They are quick responders and contribute to elimination of pathogens and tissue homeostasis by producing a variety of cytokines. Based on their specific cytokine secretion, ILCs are grouped into different classes that resemble their T-cell counterparts, for example, group 3 ILCs (ILC3s) resemble Th17 cells and produce IL-17 and IL-22 [109].

Box 1. *Overview of immune cell populations involved in intestinal antitumour immune responses.*

3. Microbial metabolites that shape antitumour immunity

Metabolites produced or modified by bacteria significantly impact health and disease by acting locally on GI tract cells but can also have systemic effects by influencing the function and activation states of immune cells. The 'metabolome' constitutes the sum of small molecules produced by a biological system and is a powerful approach to explore the current condition of that system [55]. Metabolomics refers to the analysis of metabolites using techniques, such as mass spectrometry, nuclear magnetic resonance analysis, high-performance liquid chromatography and gas chromatography coupled with mass spectrometry. Obtained peak patterns can be compared against spectral databases for identification of metabolites. Metabolomics can be combined with metagenomics, investigating the genetic material of the entire community, and metatranscriptomics, exploring which genes are expressed, to increase our understanding of microbiomes. The benefits, disadvantages and technical challenges of these omics techniques are reviewed extensively elsewhere [56–59].

In the following sections, we will discuss the impact of microbial metabolites on immune cell function, focusing on how these metabolites shape the immune response. The anatomical components of the intestinal immune system including immune and epithelial cell populations and the mechanisms employed by these cell populations to discriminate between commensal and pathogenic bacteria have been reviewed extensively recently [60–66].

3.1 Metabolites produced by bacteria from dietary components

3.1.1 Short-chain fatty acids

Short-chain fatty acids (SCFAs) are 1–6 carbon volatile fatty acids which can either be in straight or branched chain conformation [67]. They are end products of fermentation of indigestible carbohydrates such as starch and fibre, by anaerobic microbiota in the caecum and large intestine [68]. SCFAs are the most abundant metabolite in the colon and consist almost entirely of acetate (C2), propionate (C3) and butyrate (C4) [69]. Acetate is the most common SCFA (60% of total SCFAs) in the colon and can also reach the systemic circulation after absorption from the GI tract. Propionate and butyrate make up roughly 20% of the SCFAs in faeces each [68, 70]. Propionate is mainly metabolised in the liver after draining into the portal vein after absorption from the gut mucosa, while butyrate is the preferred energy source of colonocytes and is digested locally [71].

SCFAs affect host physiology and pathology through a multitude of local and systemic mechanisms of action (**Figure 1**). In the GI tract, they act through binding to transmembrane G protein-coupled receptors (GPRs) and diffusion into epithelial and immune cells where they modify post-translational gene expression and function as energy source.

GPRs implicated in SCFA signalling are free fatty acid receptors GPR41, GPR43 and GPR109a. The SCFAs acetate, propionate and butyrate have differing selectivity for these receptors with all three binding to GPR43, expressed on the GI epithelium and immune cells [72, 73]. Propionate and butyrate bind to GPR41, expressed by lamina propria cells in the large intestine, immune cells and cells of the peripheral nervous system [72]. Butyrate has also been found to ligate GPR109a expressed by large intestinal epithelium and certain subsets of immune cells [73].

Activation of the GPRs leads to changes in intracellular potassium concentrations [K^+], which directly activate intracellular danger-sensing molecular complexes, called inflammasomes. Integral components of inflammasomes are Nod-like receptors, which are cytosolic pattern recognition receptors (PRRs). Particularly changes in the NLRP3 and NLRP6 inflammasomes (containing Nod-like receptors 3 and 6, respectively) have been implicated in exacerbating intestinal inflammation [74–76]. Inflammasome complexes can be activated through a two-step process. The first signal is considered the 'priming signal', which induces nuclear factor (NF)-kB-mediated transcription of inflammasome components and pro-IL-1β and IL-18 in epithelial and immune cells [77]. The second signal leads to the assembly of the inflammasome complex and caspase-1-dependent processing of pro-IL-1β and IL-18 into their biologically active forms.

IL-1β and IL-18 are important signalling molecules for gut homeostasis and immune effector function. IL-1β can have pro- and anti-homeostatic functions, whereas IL-18 is generally regarded as a crucial cytokine for maintaining gut barrier integrity and a healthy microbiome composition. A reduction in IL-18 secretion has been found to be associated with a shift in microbiota towards the expansion of *Bacteroidetes*, which promote colonic inflammation and carcinogenesis in mouse models [46, 76]. This aligns with findings that describe a decreased

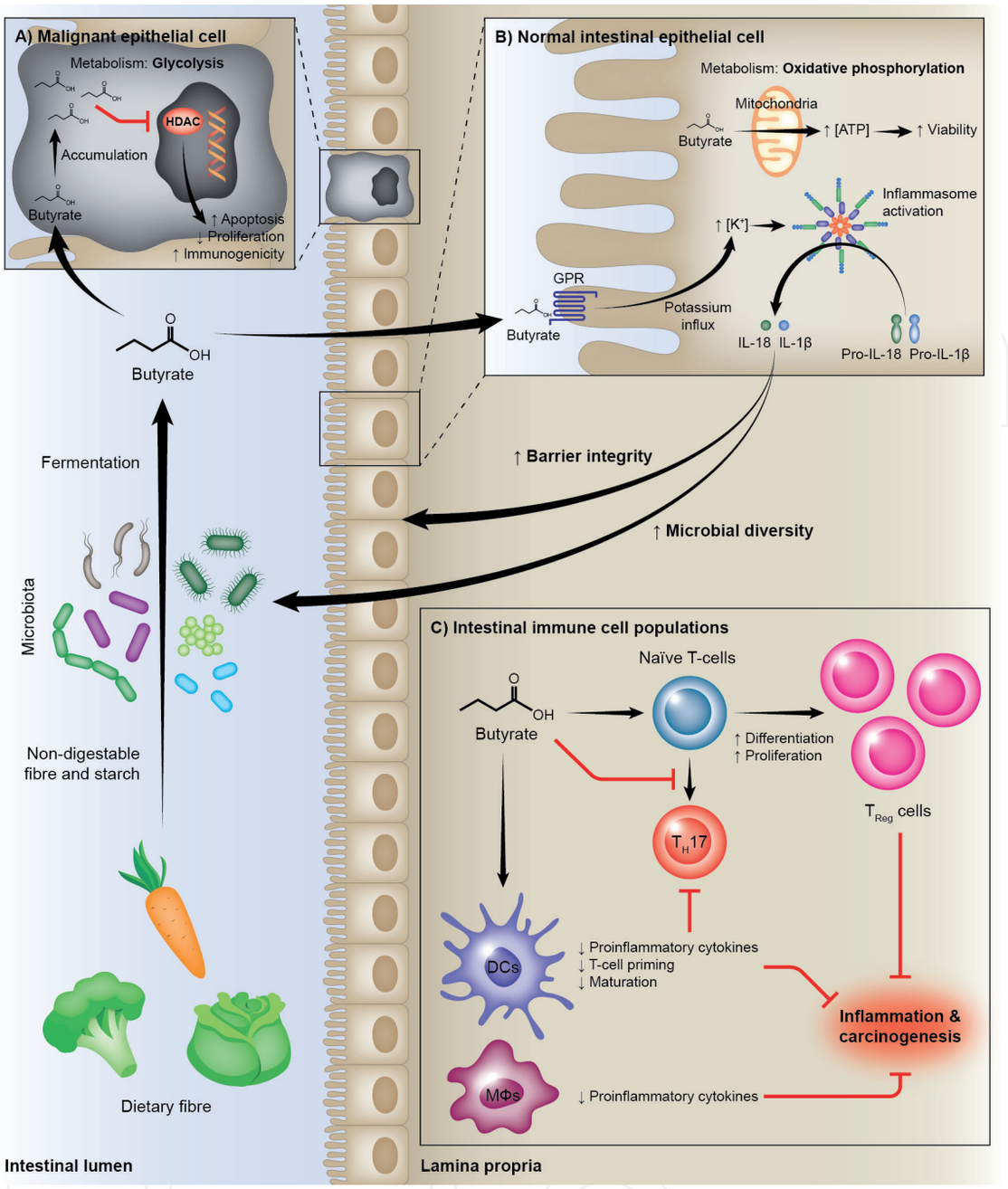

Figure 1.
Effects of the SCFA butyrate on epithelial and immune cell function. SCFAs are produced through fermentation of non-digestible fibre and starch by microbiota. (a) Cancer cells switch their metabolism to glycolysis and are less efficient at metabolising SCFAs such as butyrate, leading to accumulation of butyrate in the cell. Increased concentrations of butyrate inhibit HDAC activity and induce apoptosis, reduce proliferation and increase immunogenicity of cancer cells. (b) In healthy epithelial cells, butyrate is metabolised through oxidative phosphorylation and used as energy source by the cell. Butyrate also activates NLRP3 and NLRP6 inflammasomes through binding to GPRs, resulting in secretion of cytokines IL-1β and IL-18. In turn, IL-18 strengthens intestinal barrier integrity and promotes diversity of intestinal microbiota. (c) The effects of butyrate on immune cells in the lamina propria can be described as promoting the development and activity of anti-inflammatory populations, such as Tregs, while suppressing immune cell functions contributing to inflammation. Butyrate suppresses the maturation of DCs, limits their ability to prime CTLs and reduces the production of pro-inflammatory cytokines in DCs and macrophages. Together this reduces inflammation and the development of inflammatory Th subsets, such as Th17, which contribute to intestinal carcinogenesis.

expression of NLRP6 in gastric cancer, correlated with a reduced survival time in patients [78]. When the NLRP6 inflammasome was overexpressed, gastric cancer cell proliferation and development were inhibited, and migration and invasion of cancer cells were decreased. Furthermore, NLRP6 activity has been linked with intact epithelial barrier function and prevention of colorectal cancer

development [79]. Even though NLRP3 inflammasome activation has been shown to contribute to tumour-promoting inflammation and immune infiltrate in several ways [11, 80–83], many reports highlight the beneficial functions of the NLRP3 inflammasome in preventing intestinal cancer development. For example, activation of NLRP3 inflammasomes has been demonstrated to protect from intestinal carcinogenesis via IL-18-mediated epithelial repair [84] and suppression of metastatic colon cancer growth via maturation of natural killer (NK) cells and stimulation of their tumoricidal activity [85]. The complex biology of intestinal inflammasome signalling and its role in tumorigenesis have been reviewed recently [74, 86]. It remains to be investigated how the often overlapping and controversial findings regarding inflammasome functions orchestrate induction and resolution of inflammation.

SCFAs, particularly butyric acid and β-hydroxybutyrate, stimulate NLRP3 and NLRP6 inflammasomes through binding to GPR43 and GPR109a, leading to increased production of IL-1β and IL-18 [87, 88]. Subsequently it was shown that dietary supplementation with sodium butyrate or increased consumption of dietary fibre protected mice against colitis [87] and colonic carcinogenesis [89] through production of IL-18 and promotion of gut homeostasis. Interestingly, even though activation of the NLRP3 inflammasome was mediated via activation of GPRs, stimulation of GPRs with synthetic agonists did not recapitulate these findings, indicating that SCFAs must act on additional targets that influence cytokine secretion [90].

A prominent target of SCFAs is histone deacetylases (HDAC) and acetyltransferases (HAT), which regulate gene expression by allowing or preventing access of the transcription machinery to DNA. HDAC inhibitors have been used in cancer therapy for their ability to induce cancer cell death, reduce proliferation and increase immunogenicity of cancer cells as well as stimulate anticancer immune function [91–93]. Cancer cells utilise glucose as their primary energy source, and thus SCFAs, such as butyrate, accumulate and due to increased concentration inhibit HDAC activity [94]. In contrast, healthy cells are capable of metabolising butyrate into small molecules required for energy generation, thereby preventing accumulation of butyrate and HDAC inhibition [95, 96].

Interestingly, a similar mechanism may explain the diverging effects of butyrate on immune cell populations in the gut. In order to retain intestinal homeostasis, immune cells have to remain passive when challenged with host microbiota and food antigens yet remain responsive to fight pathogenic bacteria. This diversity of function is supported by SCFAs that induce a hypo-responsive state in immune cell populations, which are capable of promoting inflammation, such as macrophages, dendritic cells (DCs) and T cells [47], yet cells involved in containing inflammation are induced and expanded by SCFAs [97, 98].

DCs and macrophages are professional antigen-presenting cells, highly proficient at scanning the environment for invaders or tissue disturbances. Once detected, pathogens or abnormal cells are engulfed, processed and presented in small fragments to T helper (Th) cells. These cells, in turn, differentiate into populations of effector Th cells, directed by cytokines from DCs. Secretion of pro-inflammatory cytokines by DCs, such as IL-6, IL-12 and IL-23 in particular, supports the polarisation of Th cells towards effector and inflammatory subsets Th1 and Th17, respectively. This is important for removal of pathogens but can be detrimental for tissue homeostasis if not regulated tightly. Th cells also facilitate the full activation and memory development of cytotoxic T cells (CTLs), which are able to kill antigen specifically and react swiftly in the case of a second encounter. SCFAs, butyrate and propionate, but not acetate, have been shown to reduce production of pro-inflammatory cytokines, such as IL-12 and IL-23, and chemokines in DCs and

also impair the maturation of DCs [90, 99, 100]. Changes in cytokine secretion are associated with impaired ability of DCs to prime CTLs [101], reduced polarisation of Th-cell subsets towards effector and inflammatory subsets and induction of regulatory T cells (Tregs) [102].

Regulatory T cells have an important role in control of inflammation. Tissue inflammation and autoimmunity are promoted if Tregs are not present or dysfunctional. In contrast to many other cancers where Tregs are thought to suppress effective antitumour immunity, GI cancer patients benefit from the presence of Tregs in the tumour microenvironment [103]. Tregs limit inflammatory processes, induce tolerance towards food and microbial antigens and promote stem cell renewal in the intestine through a variety of mechanisms. This includes production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor beta (TGF-β)1, expression of inhibitory molecules and restriction of nutrients required by effector T cells, particularly Th1 and Th17 cells [64, 104–106].

Interestingly, due to the high rates of glycolysis in effector and pro-inflammatory cells, such as CTLs, Th1 and Th17 subsets, butyrate accumulates in these cells, leading to an inhibitory effect mediated by both HDAC inhibition and binding to GPR109a [90, 93, 102]. In contrast, anti-inflammatory cells, such as Tregs, which rely on oxidative phosphorylation can process butyrate for energy consumption, circumventing these effects [107]. It has been demonstrated comprehensively that SCFAs drive Treg development via HDAC inhibition and GPR activation in the intestine and periphery, thereby protecting mice against colonic inflammation, colitis and colorectal cancer [97, 98, 106, 108].

3.1.2 Indole derivatives

Indoles are aromatic heterocyclic compounds, produced by gut bacteria from the degradation of tryptophan via several enzymes [109]. Tryptophan is an essential amino acid, which cannot be produced by the host and is taken up in the diet. Dietary tryptophan can be metabolised by microbiota and host cells to indole derivatives that have important immune modulatory functions in the gut [110]. Indole derivatives, such as kynurenines, are ligands for the aryl hydrocarbon receptor (AHR), an intracellular ligand-activated transcription factor with important roles in detecting environmental changes and alerting cells to them. Microbial AHR ligands are thought to play an important role in maintaining intestinal homeostasis and limiting inflammation [111]. The importance of AHR signalling has been demonstrated in $AHR^{-/-}$ mice where clearance of pathogenic bacteria was impaired while intestinal inflammation was elevated and associated with an increased risk of developing colitis [112–114].

Mechanistically, bacterial AHR ligands have been shown to induce the production of IL-22 in innate lymphoid cells (ILCs), which promotes diversity of gut microbiota and protects mucosal barrier functions [115, 116]. ILCs stem from the lymphoid lineage but have innate immune cell characteristics [117]. They are quick responders and contribute to elimination of pathogens and tissue homeostasis by producing a variety of cytokines. Based on their specific cytokine secretion, ILCs are grouped into different classes that resemble their T-cell counterparts, for example, group 3 ILCs (ILC3s) resemble Th17 cells and produce IL-17 and IL-22 [117]. Even though production of IL-22 by ILC3s is vital for mucosal homeostasis, elevated levels of ILC3s and increased production of IL-17 have been associated with IBD pathology [118, 119]. Furthermore, it has been found that IL-22 contributes to tumorigenesis in the colon when elevated chronically. This was mediated via an inflammasome-dependent reduction of IL-22 binding protein and chronic elevated IL-22 levels [50, 51].

Genetic induction of constitutively active AHR signalling in mouse models has been found to be associated with stomach and liver cancer development [120, 121], whereas the absence of AHR in $AHR^{-/-}$ mice protected from prostate cancer [122]. As AHR is crucially involved in early development, maintenance of stem cells and cell differentiation, it is difficult to discern if stable genetic induction or ablation of these signalling pathways may promote carcinogenesis directly or through disturbances in early development.

3.1.3 Polyamines

Polyamines are small polycationic molecules, derived either from the diet or synthesised by gut bacteria or host cells [123]. While they are found in almost all living cells, the method of production in mammalian and bacterial cells differs. Intestinal bacteria use inducible or constitutive forms of amino acid decarboxylase enzymes in order to produce polyamines with arginine as a precursor. Mammalian synthesis involves a series of steps to convert arginine to polyamines, with ornithine decarboxylase being the rate-limiting enzyme. Putrescine, spermidine and spermine are the major polyamines secreted by both the gut microbiota and mammalian cells and have important immune modulatory functions [124].

Along with other polyamines, spermine directly regulates cells in the innate arm of the immune system and has an anti-inflammatory effect. Spermine inhibits lipopolysaccharide-induced expression of pro-inflammatory cytokines in monocytes and macrophages [125]. In macrophages, spermine is able to increase the expression of IL-10 and suppress production of inflammatory cytokines such as IFN-γ [126]. These functions were shown to have anti-inflammatory and protective effects in animal models of local and systemic inflammation [127].

Conversely, spermine inhibits the activation of the NLRP6 inflammasome and reduces the amount of IL-1β and IL-18 released. This is counteracted by taurine, another microbial metabolite, which is discussed below. The inhibitory effect of spermine on NLRP6 activity may be counteracted by the role polyamines play in maintenance of the gut epithelial lining. Many studies have found that intestinal mucosal repair is associated with an increase in levels of spermine, spermidine and putrescine [128]. Furthermore, when the synthesis of polyamines is blocked, migration and proliferation of intestinal epithelial cells to the site of injury as well as in regular turnover of mucosal cells are significantly reduced. Polyamines promote the transcription of E-cadherin, which is important for the formation of tight junctions. In this regard, they play a role in stabilising the gut epithelium, so it is able to act as a barrier between the external and internal environment [128].

3.2 Metabolites modified by bacteria, derived from host products

3.2.1 Bile acids and derivatives

Bile acids are physiological surfactants, produced in the liver and secreted into the duodenum or stored in the gall bladder. Bile acid molecules contain a hydrophobic hemisphere and a hydrophilic one, enabling them to associate around dietary fats and fat-soluble vitamins into micelles [129]. This promotes the breakdown and absorption of these molecules in the hydrophilic environment of the GI tract [130]. Approximately 95% of bile acids are reabsorbed via active transport by the apical sodium-dependent bile acid transporter in the ileum of the small intestine [131]. Microbial bile salt hydrolases catalyse the hydrolysis of amide bond linkage in bile acids, releasing an unconjugated bile acid. The de-conjugation of bile acids causes the release of glycine and taurine, which can then be used for further metabolism and growth.

Bile acids modulate innate immune cell function by inhibiting NF-kB activity, resulting in reduced production of pro-inflammatory cytokines and molecules (TNF-α, IL-1β, IL-6, IL-12, cyclooxygenase-1 and cyclooxygenase-2, and inducible nitric oxide synthase) in stimulated monocytes, macrophages, DCs and intestinal epithelial cells [132–135]. In human macrophages, administration of bile acids leads to increased production of IL-10 and a decrease in phagocytosis [136]. The reduction in pro-inflammatory cytokines combined with the increase in anti-inflammatory cytokine production induces the development, recruitment and expansion of Tregs in the colon. Together, the properties of bile acids improve barrier integrity and outcomes in mouse models of experimental colitis, which lead to the development of inflammatory bowel disease and colorectal cancer [133]. The effects occur via bile acid-mediated activation of the farnesoid X receptor (FXR), a ligand-activated nuclear receptor, and the G protein-coupled bile acid receptor 1 (GPBAR1). These receptors also play a crucial role in bile acid-induced inhibition of the NLRP3 inflammasome, which is associated with reduced levels of secreted IL-1β and IL-18 [137, 138].

In contrast to primary bile acids, the bile acid-derivative taurine stimulates NLRP6 inflammasome activity, leading to increased production of IL-18 [139]. Levy et al. found high taurine and associated IL-18 concentrations maintained and restored functional microbiota typically present in healthy flora.

3.3 Metabolites synthesised by bacteria directly

Microbiota are able to synthesise metabolites that are either unique to prokaryotic organisms, such as capsule polysaccharides and certain vitamins, or that can also be produced by host cells, for example, adenosine triphosphate (ATP).

3.3.1 ATP

In addition to its role as universal energy source, ATP is an important signalling molecule that directly impacts immune cell function when released into the extracellular space. ATP is not only produced by living organisms but has been found to be secreted by a variety of commensal and pathogenic bacteria [140, 141]. Generally, increased levels of ATP are produced and secreted by host cells under inflammatory stress conditions and injury, often associated with inflammatory cell death [142]. Furthermore, the tumour microenvironment has high concentrations of extracellular ATP, at least partly induced by hypoxia, an activator of ATP secretion, and necrotic cell death [143, 144]. The chronic presence of ATP in the tumour microenvironment supports cancer cell proliferation, survival and metastasis as reviewed elsewhere [144]. In the immune context, most of the actions of ATP have been described to be pro-inflammatory; however, its hydrolysis product adenosine has immune-suppressive functions.

Host-, tumour- and microbial-derived ATP binds to purinergic-type receptor P2, while adenosine, the downstream product of hydrolysed ATP, binds to P1 receptors [144]. Purinergic P2 receptors are expressed highly by immune cells, and ATP exerts most of its pro-inflammatory effects through binding to P2X(1–7) ion channels and P2Y(1, 4, 6, 11–14) metabotropic purinergic receptors [145]. Activation of purinergic receptor P2X7 by ATP increases intracellular potassium and calcium concentrations [146]. Together with a priming signal, ATP is an important inducer of NLRP3 inflammasome activity [147]. As discussed previously, activation of the NLRP3 inflammasome and the subsequent secretion of IL-1β and IL-18 have important roles in shaping the magnitude of inflammatory responses, gut homeostasis and barrier function and have a controversial role in tumour progression [86].

Besides its role as inflammasome activator, ATP modulates migration of innate and adaptive immune cell subsets [148]. After release of ATP into the extracellular space, innate immune cells such as monocytes, mature DCs, neutrophils, macrophages and microglia are mobilised via activation of P2X and P2Y receptors and migrate to the source of the high ATP concentration. This migratory response is further amplified through autocrine activation of pannexin 1 channels in the membrane of innate immune cells [149–153]. Interestingly, ATP has been shown to affect migration of $CD4^+$ T-cell subsets differently, depending on their function and activation status. While activated $CD4^+$ T cells respond to high ATP concentrations and stimulation of P2X7 and P2X4 receptors with induction of apoptosis, immune-suppressive Tregs increase proliferation and migration via their P2Y2 receptor [154].

In the context of intestinal inflammation and carcinogenesis, ATP drives the polarisation of $CD4^+$ T cells towards IL-17-producing $CD4^+$ T cells, associated with a higher susceptibility to develop colitis and exacerbation of existing colitis in experimental mouse models [155, 156]. A Th17 signature in colorectal cancer patients is associated with disease progression and worse outcomes [53]. Polymorphism of the ATP-converting enzyme CD39 (hydrolysis of ATP to adenosine diphosphate (ADP)) in IBD patients and increased expression of P2X7 receptors in the inflamed epithelium of Crohn's patients have been found, suggesting another role of ATP in disease pathology [156, 157].

ATP is hydrolysed by CD39 and CD73 to adenosine, which have been widely investigated and reviewed for their immune-suppressive functions in the tumour environment [144, 151, 152, 158]. Therapeutic inhibition of ATP and adenosine receptors as well as targeting of CD39 and CD73, alone and in combination with traditional chemotherapy, has shown great promise to prevent tumour growth by overturning adenosine-induced immune suppression [159–163]. However, recent evidence demonstrates that extracellular ATP is required for the formation of long-term, antigen-specific CTL responses, which are crucial for immunological memory [164]. It remains to be determined if therapeutic targeting of purinergic receptors and conversion enzymes affects development of immunological memory in cancer, which is desirable to prevent cancer occurrence.

3.3.2 Vitamins

Humans lack the ability to produce most essential vitamins and rely on vitamins to be supplied with the diet and produced by gut bacteria. Microbiota are able to synthesise vitamin K and a large number of B vitamins, such as folate (vitamin B9), riboflavin (vitamin B2), pyridoxine (vitamin B6), cobalamin (vitamin B12) and methionine [165]. B vitamins have achieved great attention for their cancer-preventing properties, with folate being the most investigated B vitamin in the cancer context [166]. The cancer-preventing mechanisms have been attributed to the role of B vitamins as cofactors in metabolic processes related to energy generation and gene regulation [166, 167]. Folate (B9) and pyridoxine (B6) have also been found to modulate intestinal immunity by increasing $CD4^+$ T-cell proliferation, trafficking and survival of Treg subsets and NK cell cytotoxicity [168–170].

Interestingly, bacteria that synthesise vitamins B2 and B9 are recognised by mucosa-activated invariant T (MAIT) cells. MAIT cells reside at mucosal surfaces in the lung and the intestine [171] and are also widely distributed in tissue and the systemic blood circulation [172, 173]. They have innate immune cell features but also express a semi-invariant T-cell receptor, which can recognise antigens presented on a monomorphic MHC class 1-related protein (MR1) expressed by

antigen-presenting cells [174]. MAIT cells are first responders to a variety of infections caused by bacteria, fungi and viruses through detection of microbial B vitamin antigens.

Upon activation, MAIT cells are able to proliferate and produce cytotoxic molecules, capable of destroying infected cells displaying microbial B vitamin antigens on their MR1 protein [175–177]. Furthermore, MAIT cells produce immune modulatory cytokines, including IFN-γ, IL-2, IL-17, IL-10 and TNF-α [178]. While IFN-γ production is highly desirable to promote antitumour immunity, an IL-17 signature has been found to be associated with worse outcomes in CRC patients [53]. Numbers of MAIT cells decrease in the peripheral circulation but accumulate in intestinal tumours [179, 180]. Several groups report a diminished ability of tumour-infiltrating MAIT cells to produce IFN-γ combined with increased secretion of IL-17 [180, 181]. Even though it appears that MAIT cells may develop a tumour-promoting phenotype in the tumour microenvironment and thus contribute to cancer progression, further studies are needed to elucidate the role of these recently discovered cells.

It is tempting to speculate that MAIT cells may impact intestinal cancer development and progression through recognition of B vitamin antigens produced by dysbiotic and carcinogenic bacteria. Since MAIT cells can be activated or inhibited depending on the B vitamin antigen presented on MR1 proteins, MAIT cells have been suggested as attractive targets for cancer immunotherapy [182]. This is in part related to their potential to be targeted in combination with chemotherapy, due the expression of drug resistance proteins that allows their survival and activation during and post-chemotherapy [172].

3.3.3 Bacterial polysaccharides

Commensal bacteria contribute to intestinal homeostasis through production of capsular polysaccharides. Polysaccharide A (PSA), the most studied bacterial polysaccharide, is produced by *B. fragilis* and plays an important role in regulating intestinal inflammation. Exogenous administration or bacterial production of PSA can prevent the development of experimental colitis by activating Treg and inhibiting Th17 responses [183–185]. This is mediated by PSA binding to PRRs expressed by DCs, which in turn secrete IL-10 that promotes the development and activation of Tregs. Furthermore, PSA influences the polarisation of Th subsets towards IFN-γ-producing Th cells, an important effector population for anticancer immunity. *B. fragilis* are not the only commensals that regulate inflammation; many other strains, for example, a large number of *Clostridium strains* [186], have been shown to have anti-inflammatory functions. This highlights the fact that the sum of commensals and their metabolites, rather than a defined strain or metabolite, shapes the functionality of the immune system by impacting the polarisation of immune subsets, crucial for clearance of diseases.

4. Conclusions

The link between microbiome disturbances and the development of inflammatory diseases highlights the importance of studying the effects of microbes and their metabolites on immune cell function. Deciphering the effects of microbial metabolites on the immune system in a highly dynamic organ system, such as the GI tract, is inherently difficult. The actions of individual metabolites need to be considered before the complex interplay of microbes, metabolites and cellular components, such as epithelial and immune cells can be investigated. The GI

tumour microenvironment is unique in that immunological tolerance is required to maintain a healthy intestinal environment, including maintenance of the "normal" microbiome, yet the presence of regulatory immune cells may impede antitumour immune responses and promote carcinogenesis.

There is increasing evidence from preclinical mouse model systems and human studies that GI tract microbiota, such as *B. fragilis*, *Bifidobacterium*, *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, can directly influence response to treatment including immunotherapies and survival in some malignancies [187–193]. This effect is potentially mediated by bacteria stimulating activation of innate immune cells and downstream polarisation of Th-cell subsets towards Th1 cells [194]. The species and diversity of bacterium identified as influencing treatment response and survival vary, likely reflecting the complexity of the interactions involved, the diverse malignancies and populations within which those malignancies had arisen, and the number of bacterial species that have immunomodulatory effects mediated through the GI tract.

The influence of infections on initiation and promotion of cancer has been long recognised, but our understanding of the complex network of interactions between the host, the microbiome, the genetics of both the host and microbiome and the metabolome remains superficial. These interactions are not static, which, with the diversity of the GI tract environment, add to the challenge of deciphering what microbial species may be influencing the immune response in a tumour-promoting or tumour-suppressive manner. The complexity of microbial species and indeed the complexity of immune cells and their function mean that practically assaying and identifying individual species of bacteria, or subsets of immune cells, clinically in a prognostic or predictive sense is challenging. The more readily measureable microbial metabolome may provide a more clinically accessible read-out of this interaction. The wide-ranging impact that products of microbial metabolism have on immune cell function and polarity and therefore anticancer immunity has been underappreciated to this point. A greater understanding of how microbial metabolites influence the GI tumour microenvironment has the potential to expand therapeutic options and improve survival of patients with GI cancers.

Conflict of interest

The authors declare no conflict of interest.

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