Regional specialization of macrophages along the gastrointestinal tract

Dorothée L. Berthold[1], Kelsey Jones[1] & Irina A. Udalova[1,*]

Author's affiliations: [1]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom

*Correspondence: irina.udalova@kennedy.ox.ac.uk

Keywords :

Macrophages, localization, microbiota, diet, transcriptional changes

Abstract

Tissue microenvironment is a major driver in imprinting tissue-specific macrophage functions in various tissues. As monocytes are recruited in numbers into the gastrointestinal (GI) tract at steady state and inflammation, they rapidly adopt a tissue-specific and distinct transcriptome. However, the GI tract varies significantly along its length, yet most of the studies of intestinal macrophages do not directly compare the phenotype and function of these macrophages in the small and large intestine, thus leading to disparity in interpretation of data between different groups. This review aims to decipher the differences along the GI tract that are likely to influence macrophage function, with a specific focus on diet and microbiota. We envisage that this analysis will fuel further investigation into the interplay of the intestinal immune system with tissue microenvironments along the GI tract, as well as provide unique therapeutic targets to modulate specific intestinal macrophage populations and/or function.

Main text

Intestinal tissue microenvironment and macrophages

The tissue microenvironment along the mammalian gastrointestinal (GI) tract differs between the small intestine (SI) and the colon, but also locally, along the crypt-lumen axis [1](see Figure 1). Along the length of the GI tract, the microbial load, mucus thickness, and pH increase from the SI to the colon [1]. The SI is critically important for nutrient absorption and food tolerance; It is also continuously exposed to digestive fluids such as bile, which can affect the microbiota that reside in the lower part of the mammalian SI [2], [3][]. The colon is heavily populated by the microbiota, which maintain a symbiotic relationship with the host and break down nutrients not taken up in the SI [4], [5]. Another important function of the colon is water resorption, which happens mainly in the proximal part of the colon [6]. Although the anatomical and physiological differences along the GI tract are extensively reviewed [7], their effects on the host immune system remain largely unexplored. The intestine reacts quickly to inflammatory changes via the fast recruitment of immune cells to relevant sites. The clear differences in the microenvironment cues along the GI tract are likely to shape the localization, phenotype and function of the resident and recruited immune cells and lead to yet unappreciated cell heterogeneity along the tract. For instance, **short chain fatty acids** (SCFAs) (see Glossary) and **bile acids (BAs),** are known to modulate the microbiota composition, but also to exert distinct effects on the intestinal immune system [8]–[11].

In this review, we focus on the impact of the changing microenvironment in the GI tract on the specialization of intestinal macrophages, which represent the largest pool of **tissue resident macrophages (TRM)** in the human body [12], [13]. The current problem in the field is that studies of intestinal macrophages mostly do not directly compare macrophages from small and large intestine, and rarely consider differences in housing and microbiota between facilities, different dietary regiments, and mouse models of intestinal inflammation, all of which impacting macrophage phenotypes. This review aims to ratify this disparity by describing the distinct environments macrophages and monocytes fate along the GI track leading to their likely differences in phenotype and function in the SI and colon.

Distinct localization of macrophages in the GI tract

Intestinal TRMs interact closely with intestinal epithelial cells (IECs), contributing to the maintenance of tissue homeostasis and barrier function [14]. Due to their position directly underneath the epithelial cell layer, macrophages are ideally equipped to capture and eliminate any microbial intruder as well as to support barrier integrity [15], [16]. In the murine SI and colon, they form a close network with the mucosal vasculature at steady state[17]. Despite their bactericidal activity and expression of a full range of **pathogen recognition receptors** (PRRs), intestinal macrophages generally remain unresponsive [15], [18]–[21]. This feature is mainly attributed to the anti-inflammatory IL10/IL10R axis in both human and mice [20], [21]. Indeed, intestinal macrophages promote tolerance towards the **microbiota** and dietary antigens; they achieve this via luminal sampling and transferring antigens to migratory dendritic cells (DC) as well as by maintaining and expanding **regulatory T cells** [22]–[25].

In most tissues, TRMs are established during embryogenesis and maintained by self-renewal. However, in the GI tract, the majority of TRMs are being replenished by recruitment of blood monocytes, caused by continuous low-grade inflammation [26], although populations of self-maintaining non-monocyte-derived macrophages in the GI tract, have also been identified by comprehensive fate mapping, single cell and bulk RNA-sequencing and imaging techniques [27] [28]. The proportions of those macrophages differ between SI and colon and the functional differences between long-lived and recruited macrophages remain to be elucidated. Embryonic progenitors and monocytes contribute to distinct macrophage phenotypes and function in micro-anatomical niches [27]. Progenitor-derived self-maintaining macrophages support the vasculature network in the LP and regulate neuronal function and motility in the muscularis externa [27]. Monocyte-derived macrophages mainly populate the villi and submucosa and are also able to fill the niche upon depletion of self-maintaining macrophages, demonstrating the niche importance in programming the macrophage phenotype and function [27].

Ly6C^{Hi} monocytes egress from the bone marrow to the blood and infiltrate the SI and colon in a CCR2-dependent manner both at steady state and inflammation in mice [19], [29]. [30]. They play a pivotal role in

driving intestinal inflammation, e.g. treatment with a CCR2-blocking antibody (MC21) resulted in an amelioration of dextran sulfate sodium (DSS)-induced colitis [30].–Moreover, the differentiation of monocytes into TRMs follows the so-called "**monocyte waterfall**" in mice, which involves the downregulation of Ly6C and subsequent upregulation of MHCII and pan-macrophage markers such as F4/80, CD64 and CX3CR1 [31]. Upon extravasation, monocytes rapidly adopt a tissue-specific **gene expression** profile [32]. Indeed, **hierarchical clustering** of **histone** epigenetic modifications has suggested that monocytes express similar genetic and epigenetic profiles to colonic and ileal macrophages, confirming that myeloid ontogeny [33]. Transcriptomic analysis of colonic monocytes-macrophages at all stages of the monocyte waterfall revealed a dependency on TGFβR-signaling for macrophage differentiation in mice [34]. TGFβ and IL10 imprint distinct gene expression profiles in murine colonic macrophages, suggesting complementary roles in shaping tissue-maintenance functions and non-responsiveness to TLR stimulation. [34]. Different subsets of macrophages exist in the steady state GI tract, distinct in function and localization [27], [35] (see Box 1). Moreover, within colonic macrophages two subsets have recently been identified in mice: $CD11c^+$ macrophages are localized close to the tip of the villi and express a pro-inflammatory transcriptome, whereas $CD206^+$ macrophages – characterized also by CD169 expression – locate close to the base of the crypt, fulfilling more tissue-resident functions [35], [36]. Flow cytometric analysis of single-cell suspensions from mucosa of duodenal-proximal jejunum (human SI) also revealed the presence of $CD11c^+$ and $CD11c^-$ macrophages, with $CD11c^+$ being short-lived monocyte-derived cells [37]. Their numbers selectively expanded in the intestinal mucosa of celiac disease patients after gluten challenge [38], but also in the inflamed colon from patients with inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC) [39],suggesting that $CD11c^+$ monocyte-macrophages are increasingly recruited to both human SI and colon during local inflammation.

The tissue microenvironment can shape macrophage identity

Fate-mapping has been extensively relied on to identify lineage specificity of macrophage subsets in different murine tissues [27], [40]. Using a monocyte transfer approach to fate-map the development of hematopoietic stem cell-derived monocytes in the murine SI, distinct mechanisms of recruitment and gene

expression at steady state and during sterile inflammation were unraveled [29]. For example, both the chemokine receptor CCR2 and the β7-integrin were deemed essential for monocyte recruitment to the SI under homeostatic conditions, based on competitive monocyte transfer experiments with wild type and respective knockout, but β7-integrin was dispensable in a sterile inflammatory setting [29]. Instead of differentiating gradually into TRMs**,** upon inflammation monocytes adopt a different transcriptomic expression pattern immediately upon entry into the SI [29]. Moreover, comparison of the gene expression of transferred monocytes into the murine SI to monocytes in other tissues, supported the concept that the tissue microenvironment is crucial for monocyte to macrophage differentiation and development [29].

Another recent study followed monocyte differentiation in the GI track after depletion of TRMs and retrieved the grafted SI and colon monocytes at different timepoints post transfer [32]. Specifically, bulk RNA-sequencing revealed that ileal and colonic monocytes clustered distinctly at day four post-transfer into donor mice. Their gene expression did not change during the time observed (up until day 12 post transfer), indicating that the majority of changes in the monocytic transcriptome occurred immediately after extravasation [32]. However, engrafted monocytes differed in their transcriptomic profile from resident macrophages, possibly due to insufficient time allowed for acquiring the full gene expression of resident cells [32]. Monocytes rapidly acquired tissue-specific characteristic transcriptomic signatures upon their entry of distinct gut segments, highlighting the importance of comparing functional differences of macrophages along the GI tract. Of note, the expression of various transcription factors (TFs) differs in monocytes of the SI and colon at steady state including, *Klf4, Foxp1* and *Trim16* for colon macrophages, and *Elk3, Hoxb3* and *Gata5* for SI macrophages [32] (see Figure 2). Whether these TFs orchestrate the expression of down-stream genes remains to be elucidated. A more detailed identification of tissue-specific transcription factors in the GI tract in mice and especially humans may help us to better understand the homeostatic functions that a macrophage is performing, and ideally, also inform on how to modulate their function under inflammatory conditions.

Functionally, the GI tract is central for nutrient digestion, partially relying on the microbiota to break down nutrients otherwise inaccessible to mammals. Nutrients taken up by diet as well as secondary metabolites and the microbiota itself might therefore have significant impact on macrophage function. This review will further focus on delineating the differences of dietary nutrients and microbiota along the GI tract as well as discuss known and possible implications on macrophage function.

Diet influences macrophage function

The luminal concentration of diet-derived **immunomodulators** depends on dietary intake but is modulated by the extent of in-lumen metabolic processing/degradation and depletion by absorption [41]. While some intestinal macrophages may be able to sample the luminal contents directly, local nutritional immunomodulation of macrophages will generally require nutrient absorption to the lamina propria or may occur as a secondary consequence of altered epithelial cell function/signaling. Because metabolic and absorptive processes, and epithelial functional responses, are spatially restricted along the gastrointestinal tract for many nutrients, local and regional nutrient bioavailability may contribute regional specialization cues for macrophages (see Figure 3).

Macrophages in the Small intestine: There may be microbiota-dependent and microbiota-independent regulation of immune cells in the intestine[17], [42]. Due to reduced microbial density in the SI, diet-derived nutrients are likely to represent the dominant exogenous modulators of immune system structure and function [43], [44]. Key nutrients influencing immune homeostasis in the SI include vitamin A and aryl hydrocarbon ligands [43]. Vitamin A metabolites can have diverse effects on the intestinal immune system; They are absorbed in the SI and are present in SI tissues at high concentrations, even during dietary vitamin A deficiency (VAD), when they are fortified by reabsorption of hepatic retinyl esters mobilized in bile in mice [45], [46]. To dissect the effect of specific metabolites on various functions of the host (immune) system, the use of specialized diets is one of the gold-standard methods in the field. In mice fed on a vitamin A rich diet, vitamin A helps to maintain SI macrophages in a quiescent state with reduced expression of pro-inflammatory mediators, but also increased **Dectin-1** expression, perhaps conferring a poised configuration with

enhanced inflammatory responses to pathogen challenge (Dectin-1 agonist) [47]. Vitamin A metabolites may be delivered to tissues in the blood stream, but several lines of evidence point to the local luminal availability of vitamin A playing an important role. Intestinal DC differentiation is compromised during most prominently in the proximal SI, but recovers with oral retinoic acid supplementation over a rapid time course further pointing to local provision [48]. This shows the non-redundant role of vitamin A as an immunomodulator in imprinting tolerogenic macrophage function in the SI.

From another angle, signaling via the **aryl hydrocarbon receptor** (AHR) has been shown to be crucial for maintenance of homeostasis and modulation of antigen-presenting cell function in response to environmental cues [49], [50]. AHR ligands can be found in dietary compounds, microbial virulence factors, tryptophan metabolism as well as derivatives from the microbiota, and they are likely to be present at greater density in the upper murine SI compared to the colon based on CYP1A1, which is an enzyme regulating AHR signaling [49]. AHR recognizes both dietary components and microbiota-derived factors, and its depletion specifically in $CD11c^+$ cells enhanced susceptibility to murine colitis, indicating a regulation of epithelial homeostasis via AHR signaling in macrophages and DCs [50].

Another study highlighted the importance of enteral nutrient provision in macrophage development in the GI track. For instance, total nutrient withdrawal from the intestinal lumen in mice (during a period of total parenteral nutrition), resulted in reduced frequency of $F480^+CD11b^+$ macrophages in the SI and reduced IL-10 production by these cells relative to control mice, indicating that SI macrophages were directly regulated by dietary amino acids [44]. Moreover, this was phenocopied by provision of a protein-free diet and by treatment with rapamycin, suggesting that luminally available amino acids can shape SI macrophage homeostasis [44], although the precise mechanism by which this is achieved are yet to be determined.

Macrophages in the Colon: SCFAs are generated by the colonic microbiota via fermentation of indigestible dietary fiber and have several important impacts on macrophage function [51]. SCFAs are highest in the ascending and transverse region of the human colon, as measured in post-mortem individuals [52]. Butyrate and niacin are capable of inducing IL-10 by activating the GPR109a receptor in murine intestinal

macrophages [53]. Moreover, butyrate imprints an antimicrobial program in human intestinal macrophages via inhibition of histone deacetylase 3 (HDAC3) [11]. Low amounts of SCFA are directly associated with several inflammatory diseases [51]. Murine HDAC3 has recently emerged as a critical node for integration of host/microbiota/nutrient signals since commensal metabolism of another nutrient, phytate, to produce inositol-1,4,5-triphosphate (IP3) increases HDAC3 activity in intestinal epithelial cells [54]. whether an equivalent impact exists on macrophage function remains to be determined. Furthermore, depletion of SCFAs during antibiotic treatment leads to hyperresponsiveness of colonic macrophages resulting in a long-term increase in inflammatory T helper 1 (Th1) cell responses and sustained dysbiosis in mice [55]. Supplementation of antibiotics with butyrate prevented antibiotic-associated immune dysfunction [55]. Interestingly, the uptake of SCFAs is a defining characteristic of the long-lived Tim4+ CD4+ colonic macrophages [56]. Therefore, supplementing the diet with SCFAs might provide an elegant way to modulate macrophage function in disease. However, the precise mechanisms and regional differences in both human and mice need to be fully elucidated.

Another major player in the GI tract are BAs, secreted by the liver as the end product of cholesterol metabolism and employed in fat digestion. Furthermore, BAs are a substrate for many bacterial enzymes, leading to the conversion of primary BAs to secondary BAs [57]. They have also been shown to have an effect on microbial growth and excessive fat consumption can lead to microbiota perturbations and pathogen upgrowth in mice [58]. Entero-hepatic circulation ensures that the majority of BAs are reabsorbed by ileal IECs and transported back to the liver through the portal vein – excess BAs in the colon results in diarrheal disease [59]. Investigations into the BA pool composition of IBD patients showed a reduction of the BA pool in CD but not UC patients, pointing towards a possible mediator of differences in immune cell activation along the GI tract [60]. In both CD and UC, the percentage of primary unconjugated BAs in stool and fresh bile is increased in comparison to healthy controls, which could both be a cause or effect of inflammation and have further implications on aberrated macrophage function [60], [61]. The microorganisms responsible for BA transformations along the GI tract of mice with a defined microbiota (**gnotobiotic mice**) were identified,

using a combined metagenomics and metabolomics approach, and revealed the non-redundancy of the microbiota in shaping the BA pool composition [62]. The BA composition along the GI tract was also measured with **Liquid Chromatography-Mass Spectrometry** (LC/MS) as well as connected to the location of specific BA transformation enzymes and their associated microorganisms, providing a useful resource to map potential functions along the GI tract [62].

BAs are also are recognized directly via receptor activation by a diverse range of cells including hepatocytes, IECs, and macrophages [63]. Farnesoid receptor X (FXR) is a BA receptor that regulates homeostatic enterohepatic circulation through BA sensing in the liver and ileum [64]. Indeed, colon inflammation in human CD patients and mice has been associated with reduced expression of *Fxr* mRNA relative to homeostasis [65], [66]. Intestinal expression of *Fxr* is selectively modulated by Toll-like receptor (TLR) 9 in human monocytes and *Tlr9-/-* mice, connecting microbiota-sensing receptors to modulations of host immune and metabolic functions [67]. Furthermore, using *Fxr-/-* mice, it was shown that colon homeostasis was aberrated and progression to colonic inflammation was exacerbated [65]. Treatment of human macrophages *in vitro* with taurolitocholic acid (TLC) in the presence of LPS, reprogrammed pro-inflammatory macrophages by repressing gene expression associated with mediation of pro-inflammatory effects, phagocytosis, pathogen interaction and recruitment of other immune cells relative to stimulation with LPS alone [68]. This, albeit conducted *in vitro*, shows a direct mechanistic link between BA and modulation of macrophage function in the presence of inflammatory stimuli. TLC is one of the strongest agonists for G-protein coupled BA receptor 1 (TGR5), a secondary BA receptor which is expressed on human macrophages [69], [70]. Mechanistically, different types of BAs can induce cAMP activation via TGR5 which subsequently leads to PKA-kinase activation and resulting inhibition of LPS-induced cytokine production in primary human macrophages [71]. TGR5 is more highly expressed in the colon and may therefore contribute to distinct macrophage function along the GI tract via BA signaling. Lack of this receptor exacerbated DSS and TNBS colitis in mice in a macrophage-dependent manner [72]. Activation of TGR5 with BAR501, a small molecule agonist, in human and murine macrophages promotes a shift towards an IL-10 producing macrophage phenotype [72]. Deoxycholic acid, a secondary BA,

promoted polarization of intestinal macrophages to a pro-inflammatory phenotype in a dose-dependent manner in mice, showing the direct effect BAs can have on macrophage function [73].

Mice treated with a broad-spectrum antibiotic cocktail revealed a compartment-specific impact on macrophage function [56]. Colonic macrophages were metabolically more active upon antibiotic administration, in contrast to unchanged SI macrophages [56]. Given how changes in the microbiota modulate the BA pool, it would be interesting to see whether these changes are due to a difference of the BA pool in the colon.

Spatial organization of microbiota and its impact on macrophages

The importance of the microbiota is the intestinal homeostasis is highlighted in **germ-free** (GF) mice, where the colonic macrophage pool is diminished relative to **specific pathogen-free** (SPF) mice, possibly due to reduced monocyte recruitment [74], [75]. DCs are not decreased in GF mice and macrophage numbers can be restored upon co-housing germ-free mice with SPF mice for six weeks [36]. Specifically, the microbiota has been shown to be crucial for the development of two macrophage subsets in the colon, as two major macrophage populations ($CD11c^{+}$ $CD121b^{+}$ and $CD206^{Hi}$ $CD11c^{-}$) are selectively decreased in GF mice relative to SPF mice [36]. By investigating their transcriptome on a single-cell level, effects of the microbiota on several gene pathways, including metabolism, gene regulation, host defense and adaptive immunity was revealed [36].

Furthermore, the microbiota is also crucial in imprinting a pro-inflammatory signature in IL10R-deficient colonic macrophages as determined by the impact of antibiotic treatment [76]. Using ***MyD88***-/- mice as a model for loss of innate immune signaling revealed a shift in bacterial diversity along the GI tract and an increase in SFB in the SI , uncovering the ability of the host immune system and possibly IECs in modulating microbiota composition [77]. Additionally, a dependency for antimicrobial peptide secretion on MyD88 signaling was revealed in the colon [77]. Macrophages have also been shown to modulate the microbiota in zebrafish via expression of complement factors, offering a potential two-way axis in modulating the host immune system, further adding to its complexity in identifying factors responsible for the localization of

macrophages [78]. Furthermore, spatial distribution of perivascular macrophages was modified in both germ-free and antibiotic-depleted mice in the SI and colon [17]. Their localization was not mediated in a MyD88/TRIF dependent manner nor was it restored upon addition of single Toll-like receptor (TLR) stimulants, which renders the factor by which the microbiota affects the spatial distribution unknown [17].

The spatial diversity of the microbiota has been described both along the length of the intestine as well as radially [79]. In fact, the GI tract harbors several distinct niches with different microenvironments for spatial localization of different microbiota members [1], [7], [80], [81]. Along the mammalian gastrointestinal tract, microbial load increases from the SI to the colon [7]. Few studies have directly investigated the diversity of the microbiota in different segments of the human gut, as it is technically challenging. A study in Iberian pigs looked at composition and diversity of microbiota in the SI (duodenum, jejunum, and ileum) as well as proximal and distal colon [82]. The **alpha diversity** was significantly higher in both parts of the colon, whereas the SI also bore greater variations between animals [82]. Also in C57BL/6 mice, using a 16S rRNA sequencing approach, bacterial communities taken from the stomach, SI (duodenum, jejunum, and ileum) as well as colon and feces were compared [83]. In the murine SI, rapidly dividing and facultative anaerobes are favored as transit is fast and oxygen concentration is relatively high compared to the colon [83], [84]. Oxygen availability is likely to have an impact on microbiota composition [85]. *Firmicutes* segmented filamentous bacteria (SFB) adhere tightly to epithelial cells and **Peyer's patches** (PP) in the ileum and have immunomodulatory effects in mice [86]. Macrophages isolated from PP of the murine SI lack most classic macrophage markers and IL-10 production upon stimulation *in vitro* [87]. Derived from the same monocyte lineage as confirmed by the use of **parabiotic mice**, they possess a distinct transcriptome compared to their villus counterparts, highlighting the importance of the microenvironment and potentially the microbiota in shaping macrophage phenotype and function [87].

Spatial reprogramming of the microbiota may be a common function of IBD that has been underappreciated, as bacterial delocalization during experimental colitis promotes macrophage activation in genetically susceptible mice [88]. Given how important the microbiota is in modulating the differentiation and

development of macrophages, it seems crucial to dissect the effects along the GI tract. To this end, a number of approaches have been applied, such as single cell RNA-seq analysis at different locations along the healthy human colon in conjunction with the microbiota [89]. This study relied on mucosal swabs from the cecum, the transverse and the sigmoid colon as opposed to stool samples and revealed a preferential colonization of specific bacterial species along the length of the human colon [89]. Furthermore, by focusing on the transcriptome of B and T cell subsets, a region-specific transcriptional and activation profile was uncovered. Using a **bioinformatic tool "pseudospace**", they were able to recreate the migration path of T cells to respective areas of the colon to understand gene signatures driving migration and tissue adaption of T cells [89]. However, this study was conducted in post-mortem individuals which might have an impact on the composition of the microbiome.

This pseudospace analysis may also be applied to other immune cell populations to decipher tissue adaption of macrophages in different locations. As it is difficult to analyze microbiota composition along the tract of living individuals and human biopsies having to undergo a bowel preparation procedure, the use of mice might be favorable to investigate regional differences in microbiota composition and immune cell functions along the gastrointestinal tract. However, the spatial distribution of microbiota in mice might not reflect the human GI tract accurately. The ability of transplanting human microbiota into GF mice offers possibilities of "screenshotting" a certain stage in time and assess the effects on the microbiome and host upon modulation. Furthermore, applying new techniques and simultaneous analysis of microbiota composition and transcriptomic activity of immune cells along the GI tract might offer new insights in mechanisms shaping macrophage functions and metabolic activity of the microbiota, respectively.

Concluding remarks

Microenvironment is as a major driver in shaping macrophage differentiation and function in different tissues in both human and mice. Despite it being vastly different along the GI tract, only a handful of studies have considered regional differences between SI and colon. They highlighted the differences in macrophage transcriptomes and metabolomes between SI and colon macrophages. This disparity needs to be addressed

in a systematic and comprehensive manner. Even more critical is the identification of TFs that establish a niche-specific macrophage blueprint along the GI tract (see “outstanding questions”), which so far remain largely unknown.

In recent years, much focus has been on the interplay between the microbiome and the intestinal immune system. Diet is a major factor influencing microbiota composition directly and the immune system more indirectly. Deciphering the precise mechanisms by which metabolites derived from food influence different microbiota taxa, as well as how their secondary metabolites influence the intestinal immune system, are needed to identify any putative disease mediators. for example, understanding the precise mechanisms by which BA modulate microbiota composition as well as how they act directly on intestinal cells, might be crucial to identify novel targets for therapeutic approaches in chronic intestinal diseases.

Establishing a complete map of microbial-dietary-host interaction along the GI tract in homeostasis and comparing to differences in various inflammatory settings is needed to better understand the etiology of different intestinal diseases and begin their compartment-specific targeting. To this end, techniques for imaging multiple microbiota members simultaneously along the GI tract will offer new insights into the physical and molecular interactions between the microbiota members as well as with the host immune system [90]. Spatial transcriptomics or other similar unbiased approaches, like **MALDI-MSI,** applied to gut swiss rolls to investigate host gene/protein expression along the whole GI tract via, will complement and help dissecting host-microbiome interactions [91], [92]. The task is challenging, but the new unbiased -omic approaches and advanced imaging techniques will undoubtedly pave the way.

Acknowledgements

We would like to thank Dr. Nicholas E. Illot for reading and correcting the manuscript and offering new angles and insights. Furthermore, we would also like to acknowledge Cornelia Heubergers contribution to Figure 1 by supplementing the small intestine histology picture. This work was supported by Kennedy Trust (PhD

studentship AZT00040 to DLB), the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement (KJ, MR/R008019/1) and the Wellcome Trust (Investigator Award 209422/Z/17/Z to IAU). Figures were created using biorender.com.

Figure legends

Figure 1: Overview of changes along the gastrointestinal tract. Shown are representative micrographs showing the small intestine (left) and the colon (right) from mice. The main function of the SI is absorption of nutrients whereas the colon is heavily populated by microbiota breaking down nutrients that were not absorbed in the SI. These differences in function are also reflected in the structure of the SI, being composed of villi to increase surface area for nutrient absorption. Localized above the crypts in the intestinal lumen is a thick mucosal layer which separates the host tissue from the microbiota and pathogens [93]. Oxygen, BAs, vitamin A and aryl hydrocarbon receptor ligands are decreased along the length of the GI tract whilst microbial load, mucus thickness and SCFA availability increases from SI to colon [7]. These changes contribute to different tissue microenvironments along the GI tract [94], [95]. Scalebar depicts 500 µM. Below, the arrows represent the directional concentration of oxygen, SCFAs, Vitamin A, AHR ligands, mucus, bile acids and the microbial load. Abbreviations: SCFA: short-chain fatty acids, SI: small intestine

Figure 2: Factors influencing the macrophage transcriptome in the murine SI and colon. The schematic depicts a comparison between macrophages in the small intestine and colon. The tissue microenvironment of a macrophage influences its tissue-specific transcriptome. In the SI, aryl hydrocarbon (AHR) ligands like dietary metabolites, microbiota derivates and tryptophan metabolites act on macrophages [47], [49]. Vitamin A in its retinoic acid form can also modulate macrophage function via increasing expression of Dectin-1 receptor [47]. Transcription factors (TFs) like *Elk3, Hoxb3* and *Gata5* were suggested to be SI-specific [32]. Secondary BAs and short-chain fatty acids (SCFAs) act on colonic macrophages via TGR5 and GPR109a, respectively [53], [72] . The microbiota was also shown to influence macrophage function, development, and localization; however, the exact mechanisms are unknown. *Klf4, Foxp1* and *Trim16* are colon-specific TFs for macrophages. The combination of these factors leads to a differential gene expression reflected in the

so-called ‘chromatin landscape’ [32], [33]. Open chromatin are regions accessible to transcription factors and to genes being transcribed [33]. Inserts indicate the implications of transcription factor-mediated gene transcription in open chromatin regions.

Figure 3: Diet and microbiota determining macrophage function along the mammalian GI tract. The schematic depicts a comparison between small intestine and colon in homeostasis. In the SI, various nutrients are broken down into their metabolite building blocks by various digestive fluids. Vitamin A and AHR ligands were shown to directly influence macrophage function by decreasing pro-inflammatory gene expression and by induction of metabolic clearance [47]. Primary bile acids (BAs) used for fat digestion are mainly reabsorbed in the ileum [59] . Primary BAs are converted into secondary BAs by the microbiota, which were shown to have various effects on macrophages [68], [71]–[73]. Fatty acids are also being converted into metabolically active SCFAs by the microbiota. They act on macrophages by inducing their antimicrobial program and ensuring hyporesponsiveness towards the microbiota by downregulating TLR responsiveness and induction of IL-10 production [53], [96]. The microbiota also acts directly on macrophages by regulating their perivascular localization and driving development of certain intestinal macrophage subsets [17]. Macrophages can also modulate microbiota composition in zebrafish [78]. Downward arrows indicate effects on macrophage function, upward arrows indicate potential effects of macrophages on microbiota. Abbreviations SI: small intestine, BA: bile acids, SCFA: short-chain fatty acids, TLR: Toll-like receptor, AHR: aryl-hydrocarbon receptor. Bacteria are represented as blue-green oval shapes, the epithelial cell layer is depicted as a single layer of brown cells. The mucus is shown in green, and macrophages depicted in purple/pink.

References

[1] G. P. Donaldson, S. M. Lee, and S. K. Mazmanian, “Gut biogeography of the bacterial microbiota.,” *Nat. Rev. Microbiol.*, vol. 14, no. 1, pp. 20–32, Jan. 2016, doi: 10.1038/nrmicro3552.

[2] K. B. M. Saiful Islam *et al.*, “Bile Acid Is a Host Factor That Regulates the Composition of the Cecal

Microbiota in Rats," *Gastroenterology*, vol. 141, no. 5, 2011, doi: 10.1053/j.gastro.2011.07.046.

[3] G. Kakiyama *et al.*, "Modulation of the fecal bile acid profile by gut microbiota in cirrhosis," *J. Hepatol.*, vol. 58, no. 5, pp. 949–955, 2013, doi: 10.1016/j.jhep.2013.01.003.

[4] W. WR *et al.*, "Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 10, pp. 3698–3703, Mar. 2009, doi: 10.1073/PNAS.0812874106.

[5] M. K. Malys, L. Campbell, and N. Malys, "Symbiotic and antibiotic interactions between gut commensal microbiota and host immune system," *Medicina (B. Aires).*, vol. 51, no. 2, pp. 69–75, Jan. 2015, doi: 10.1016/J.MEDICI.2015.03.001.

[6] G. J. Devroede, S. F. Phillips, C. F. Code, and J. F. Lind, "Regional Differences in Rates of Insorption of Sodium and Water from the Human Large Intestine," *Can. J. Physiol. Pharmacol.*, vol. 49, no. 12, pp. 1023–1029, 1971, doi: 10.1139/Y71-145.

[7] A. M. Mowat and W. W. Agace, "Regional specialization within the intestinal immune system," *Nat. Rev. Immunol.*, vol. 14, no. 10, pp. 667–685, 2014, doi: 10.1038/nri3738.

[8] M. X. Byndloss *et al.*, "Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion," *Science (80-.).*, vol. 357, no. 6351, pp. 570–575, 2017, doi: 10.1126/science.aam9949.

[9] R. Corrêa-Oliveira, J. L. Fachi, A. Vieira, F. T. Sato, and M. A. R. Vinolo, "Regulation of immune cell function by short-chain fatty acids.," *Clin. Transl. Immunol.*, vol. 5, no. 4, p. e73, Apr. 2016, doi: 10.1038/cti.2016.17.

[10] P. Gonçalves, J. R. Araújo, and J. P. Di Santo, "A Cross-Talk Between Microbiota-Derived Short-Chain Fatty Acids and the Host Mucosal Immune System Regulates Intestinal Homeostasis and Inflammatory Bowel Disease," *Inflamm. Bowel Dis.*, vol. 24, no. 3, pp. 558–572, Feb. 2018, doi: 10.1093/ibd/izx029.

[11] J. Schulthess, S. Pandey, and M. Capitani, “The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages,” *Immunity*, vol. 50, pp. 432–445, 2019, doi: 10.1016/j.immuni.2018.12.018.

[12] S.-L. Lee, P. M. Starkey, and S. Gordon, “Quantitative analysis of total macrophage content in adult mouse tissues. Immunochemical studies with monoclonal antibody F4/80,” *J. Exp. Med.*, 1985, Accessed: Sep. 23, 2020. [Online]. Available: http://rupress.org/jem/article-pdf/161/3/475/483974/475.pdf.

[13] D. A. Hume, V. H. Perry, and S. Gordon, “The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: Macrophages associated with epithelia,” *Anat. Rec.*, vol. 210, no. 3, pp. 503–512, 1984, doi: 10.1002/ar.1092100311.

[14] J. R. Grainger, J. E. Konkel, T. Zangerle-Murray, and T. N. Shaw, “Macrophages in gastrointestinal homeostasis and inflammation,” *Pflugers Arch. Eur. J. Physiol.*, vol. 469, no. 3–4, pp. 527–539, 2017, doi: 10.1007/s00424-017-1958-2.

[15] L. E. Smythies *et al.*, “Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity,” *J. Clin. Invest.*, vol. 115, no. 1, pp. 66–75, Jan. 2005, doi: 10.1172/JCI200519229.

[16] S. L. Pull, J. M. Doherty, J. C. Mills, J. I. Gordon, and T. S. Stappenbeck, “Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury,” *PNAS*, vol. 102, pp. 99–104, 2004, Accessed: Apr. 15, 2019. [Online]. Available: www.pnas.orgcgidoi10.1073pnas.0405979102.

[17] M. Honda *et al.*, “Perivascular localization of macrophages in the intestinal mucosa is regulated by Nr4a1 and the microbiome,” *Nat. Commun.*, 2020, doi: 10.1038/s41467-020-15068-4.

[18] L. E. Smythies *et al.*, “Inflammation anergy in human intestinal macrophages is due to Smad-induced

IκBα expression and NF-κB inactivation," *J. Biol. Chem.*, vol. 285, no. 25, pp. 19593–19604, Jun. 2010, doi: 10.1074/jbc.M109.069955.

[19] C. C. Bain *et al.*, "Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors," *Mucosal Immunol.*, vol. 6, no. 3, pp. 498–510, May 2013, doi: 10.1038/mi.2012.89.

[20] C. C. Bain and A. Schridde, "Origin, Differentiation, and Function of Intestinal Macrophages," *Front. Immunol.*, vol. 9, no. November, pp. 1–15, 2018, doi: 10.3389/fimmu.2018.02733.

[21] T. L. Denning, Y. C. Wang, S. R. Patel, I. R. Williams, and B. Pulendran, "Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses," *Nat. Immunol.*, vol. 8, no. 10, pp. 1086–1094, 2007, doi: 10.1038/ni1511.

[22] J. H. Niess *et al.*, "CX3CR1-Mediated Dendritic Cell Access to the Intestinal Lumen and Bacterial Clearance," *Science (80-.).*, vol. 307, no. 5707, pp. 254–258, 2005, doi: 10.1126/science.1102901.

[23] M. Kim *et al.*, "Critical Role for the Microbiota in CX3CR1+ Intestinal Mononuclear Phagocyte Regulation of Intestinal T Cell Responses," *Immunity*, vol. 49, no. 1, pp. 151-163.e5, Jul. 2018, doi: 10.1016/J.IMMUNI.2018.05.009.

[24] E. Mazzini, L. Massimiliano, G. Penna, and M. Rescigno, "Oral Tolerance Can Be Established via Gap Junction Transfer of Fed Antigens from CX3CR1+ Macrophages to CD103+ Dendritic Cells," *Immunity*, vol. 40, no. 2, pp. 248–261, Feb. 2014, doi: 10.1016/J.IMMUNI.2013.12.012.

[25] R. J. Cummings *et al.*, "Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs," *Nature*, vol. 539, no. 7630, pp. 565–569, Nov. 2016, doi: 10.1038/nature20138.

[26] M. Guilliams, G. R. Thierry, J. Bonnardel, and M. Bajenoff, "Establishment and Maintenance of the Macrophage Niche," *Immunity*, vol. 52, 2020, doi: 10.1016/j.immuni.2020.02.015.

[27] S. De Schepper *et al.*, “Self-Maintaining Gut Macrophages Are Essential for Intestinal Homeostasis,” *Cell*, vol. 175, no. 2, pp. 400-415.e13, Oct. 2018, doi: 10.1016/J.CELL.2018.07.048.

[28] T. N. Shaw *et al.*, “Tissue-resident macrophages in the intestine are long lived and defined by Tim-4 and CD4 expression,” *J. Exp. Med.*, 2018, [Online]. Available: http://jem.rupress.org/content/early/2018/05/22/jem.20180019.abstract.

[29] G. Desalegn, O. Pabst, and O. P. Girmay Desalegn, “Inflammation triggers immediate rather than progressive changes in monocyte differentiation in the small intestine,” *Nat. Commun.*, vol. 10, no. 1, pp. 1–14, 2019, doi: 10.1038/s41467-019-11148-2.

[30] E. Zigmond *et al.*, “Ly6Chi Monocytes in the Inflamed Colon Give Rise to Proinflammatory Effector Cells and Migratory Antigen-Presenting Cells,” *Immunity*, vol. 37, no. 6, pp. 1076–1090, Dec. 2012, doi: 10.1016/J.IMMUNI.2012.08.026.

[31] S. Tamoutounour *et al.*, “CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis,” *Eur. J. Immunol.*, vol. 42, no. 12, pp. 3150–3166, Dec. 2012, doi: 10.1002/eji.201242847.

[32] M. Gross-Vered *et al.*, “Defining murine monocyte differentiation into colonic and ileal macrophages,” *Elife*, vol. 9, pp. 1–19, 2020, doi: 10.7554/eLife.49998.

[33] Y. Lavin *et al.*, “Tissue-Resident Macrophage Enhancer Landscapes Are Shaped by the Local Microenvironment,” *Cell*, vol. 159, no. 6, pp. 1312–1326, Dec. 2014, doi: 10.1016/J.CELL.2014.11.018.

[34] A. Schridde *et al.*, “Tissue-specific differentiation of colonic macrophages requires TGFβ receptor-mediated signaling,” *Mucosal Immunol.*, vol. 10, no. 6, pp. 1387–1399, Nov. 2017, doi: 10.1038/mi.2016.142.

[35] A. L. Corbin *et al.*, “IRF5 guides monocytes toward an inflammatory CD11c + macrophage phenotype

457 and promotes intestinal inflammation," *Sci. Immunol.*, vol. 6085, pp. 1–16, 2020.

458 [36] B. Kang *et al.*, "Commensal microbiota drive the functional diversification of colon macrophages,"
459 *Mucosal Immunol.*, vol. 13, no. 2, pp. 216–229, Mar. 2020, doi: 10.1038/s41385-019-0228-3.

460 [37] A. Bujko *et al.*, "Transcriptional and functional profiling defines human small intestinal macrophage
461 subsets," *J. Exp. Med.*, vol. 215, no. 2, pp. 441–458, Feb. 2018, doi: 10.1084/jem.20170057.

462 [38] A.-C. R. Beitnes, M. Ráki, M. Brottveit, K. E. A. Lundin, F. L. Jahnsen, and L. M. Sollid, "Rapid
463 Accumulation of CD14+CD11c+ Dendritic Cells in Gut Mucosa of Celiac Disease after in vivo Gluten
464 Challenge," *PLoS One*, vol. 7, no. 3, p. e33556, Mar. 2012, doi: 10.1371/JOURNAL.PONE.0033556.

465 [39] B. D *et al.*, "Human intestinal pro-inflammatory CD11c high CCR2 + CX3CR1 + macrophages, but not
466 their tolerogenic CD11c - CCR2 - CX3CR1 - counterparts, are expanded in inflammatory bowel
467 disease," *Mucosal Immunol.*, vol. 11, no. 4, pp. 1114–1126, Jul. 2018, doi: 10.1038/S41385-018-
468 0030-7.

469 [40] S. Yona *et al.*, "Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages
470 under Homeostasis," *Immunity*, vol. 38, no. 1, pp. 79–91, Jan. 2012, doi:
471 10.1016/j.immuni.2012.12.001.

472 [41] D. Wu, E. D. Lewis, M. Pae, and S. N. Meydani, "Nutritional Modulation of Immune Function:
473 Analysis of Evidence, Mechanisms, and Clinical Relevance," *Front. Immunol.*, vol. 9, no. JAN, p. 3160,
474 Jan. 2019, doi: 10.3389/FIMMU.2018.03160.

475 [42] K. S. Kim *et al.*, "Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small
476 intestine," *Science (80-.).*, vol. 351, no. 6275, pp. 858–863, Feb. 2016, doi:
477 10.1126/SCIENCE.AAC5560.

478 [43] T. L. Morhardt, A. Hayashi, J. Y. Kao, and N. Kamada, "Regional Control of Regulatory Immune Cells
479 in the Intestine," *Curr. Pathobiol. Rep.*, vol. 6, pp. 29–34, 2018, doi: 10.1007/s40139-018-0156-z.

[44] T. Ochi *et al.*, “Diet-dependent, microbiota-independent regulation of IL-10-producing lamina propria macrophages in the small intestine,” *Sci. Rep.*, vol. 6, no. June, pp. 1–12, 2016, doi: 10.1038/srep27634.

[45] E. H. Harrison, “Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids,” *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids*, vol. 1821, no. 1, pp. 70–77, Jan. 2012, doi: 10.1016/j.bbalip.2011.06.002.

[46] E. Jaensson-Gyllenbäck *et al.*, “Bile retinoids imprint intestinal CD103+ dendritic cells with the ability to generate gut-tropic T cells,” *Mucosal Immunol.*, vol. 4, no. 4, pp. 438–447, Jul. 2011, doi: 10.1038/mi.2010.91.

[47] M. N. Erkelens *et al.*, “Intestinal Macrophages Balance Inflammatory Expression Profiles via Vitamin A and Dectin-1-Mediated Signaling,” *Front. Immunol.*, vol. 11, no. March, p. 551, Mar. 2020, doi: 10.3389/fimmu.2020.00551.

[48] R. Zeng, M. Bscheider, K. Lahl, M. Lee, and E. C. Butcher, “Generation and transcriptional programming of intestinal dendritic cells: Essential role of retinoic acid,” *Mucosal Immunol.*, vol. 9, no. 1, pp. 183–193, Jan. 2016, doi: 10.1038/mi.2015.50.

[49] C. Schiering *et al.*, “Feedback control of AHR signalling regulates intestinal immunity,” *Nature*, vol. 542, 2017, doi: 10.1038/nature21080.

[50] S. H. Chng *et al.*, “Ablating the aryl hydrocarbon receptor (AhR) in CD11c+ cells perturbs intestinal epithelium development and intestinal immunity,” *Sci. Rep.*, 2016, doi: 10.1038/srep23820.

[51] A. N. Thorburn, L. Macia, and C. R. Mackay, “Diet, Metabolites, and ‘Western-Lifestyle’ Inflammatory Diseases,” *Immunity*, vol. 40, no. 6, pp. 833–842, 2014, doi: 10.1016/j.immuni.2014.05.014.

[52] J. H. Cummings, E. W. Pomare, H. W. J. Branch, C. P. E. Naylor, and G. T. MacFarlane, “Short chain fatty acids in human large intestine, portal, hepatic and venous blood,” *Gut*, vol. 28, no. 10, pp.

1221–1227, Oct. 1987, doi: 10.1136/gut.28.10.1221.

[53] N. Singh *et al.*, "Activation of Gpr109a, Receptor for Niacin and the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis," *Immunity*, vol. 40, pp. 128–139, 2014, doi: 10.1016/j.immuni.2013.12.007.

[54] S. en Wu *et al.*, "Microbiota-derived metabolite promotes HDAC3 activity in the gut," *Nature*, pp. 1–5, Jul. 2020, doi: 10.1038/s41586-020-2604-2.

[55] N. A. Scott *et al.*, "Antibiotics induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis," *Sci. Transl. Med.*, vol. 10, no. 464, Oct. 2018, doi: 10.1126/scitranslmed.aao4755.

[56] N. A. Scott, M. A. E. Lawson, R. J. Hodgetts, and L. J. Hall, "Macrophage metabolism in the intestine is compartment-specific and regulated by the microbiota," *bioRxiv*, 2021, doi: 10.1101/2021.06.24.449804.

[57] S. Just *et al.*, "The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism," *Microbiome*, vol. 6, no. 1, pp. 1–18, Aug. 2018, doi: 10.1186/S40168-018-0510-8.

[58] S. Y. Wotzka *et al.*, "Escherichia coli limits Salmonella Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice," *Nat. Microbiol.*, vol. 4, pp. 2164–2174, 2019, doi: 10.1038/s41564-019-0568-5.

[59] J. Y. L. Chiang, "Bile acids: Regulation of synthesis," *J. Lipid Res.*, vol. 50, no. 10, pp. 1955–1966, 2009, doi: 10.1194/jlr.R900010-JLR200.

[60] G. Vantrappen, Y. Ghoos, P. Rutgeerts, and J. Janssens, "Bile acid studies in uncomplicated Crohn's disease," *Gut*, vol. 18, pp. 730–735, 1977.

[61] E. A. Franzosa *et al.*, "Gut microbiome structure and metabolic activity in inflammatory bowel disease," *Nat. Microbiol.*, vol. 4, no. 2, pp. 293–305, Feb. 2019, doi: 10.1038/s41564-018-0306-4.

[62] S. Marion *et al.*, “Biogeography of microbial bile acid transformations along the murine gut,” *J. Lipid Res.*, vol. 61, no. 11, pp. 1450–1463, Nov. 2020, doi: 10.1194/jlr.RA120001021.

[63] S. Fiorucci *et al.*, “Bile Acid Signaling in Inflammatory Bowel Diseases,” *Dig. Dis. Sci.*, vol. 1, p. 3, Dec. 2020, doi: 10.1007/s10620-020-06715-3.

[64] J. R. M. Plass *et al.*, “Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump,” *Hepatology*, vol. 35, no. 3, pp. 589–596, Mar. 2002, doi: 10.1053/JHEP.2002.31724.

[65] P. Vavassori, A. Mencarelli, B. Renga, E. Distrutti, and S. Fiorucci, “The Bile Acid Receptor FXR Is a Modulator of Intestinal Innate Immunity,” *J. Immunol.*, vol. 183, no. 10, pp. 6251–6261, Nov. 2009, doi: 10.4049/jimmunol.0803978.

[66] J. Torres *et al.*, “Farnesoid X Receptor Expression Is Decreased in Colonic Mucosa of Patients with Primary Sclerosing Cholangitis and Colitis-associated Neoplasia,” *Inflamm. Bowel Dis.*, vol. 19, no. 2, pp. 275–282, Feb. 2013, doi: 10.1097/MIB.0b013e318286ff2e.

[67] B. Renga *et al.*, “The Bile Acid Sensor FXR Is Required for Immune-Regulatory Activities of TLR-9 in Intestinal Inflammation,” *PLoS One*, vol. 8, no. 1, Jan. 2013, doi: 10.1371/journal.pone.0054472.

[68] M. Wammers *et al.*, “Reprogramming of pro-inflammatory human macrophages to an anti-inflammatory phenotype by bile acids,” *Sci. Rep.*, vol. 8, no. 1, pp. 1–15, Dec. 2018, doi: 10.1038/s41598-017-18305-x.

[69] Y. Kawamata *et al.*, “A G protein-coupled receptor responsive to bile acids,” *J. Biol. Chem.*, vol. 278, no. 11, pp. 9435–9440, Mar. 2003, doi: 10.1074/jbc.M209706200.

[70] V. Keitel, M. Donner, S. Winandy, R. Kubitz, and D. Häussinger, “Expression and function of the bile acid receptor TGR5 in Kupffer cells,” *Biochem. Biophys. Res. Commun.*, vol. 372, no. 1, pp. 78–84, Jul. 2008, doi: 10.1016/j.bbrc.2008.04.171.

[71] K. Haselow *et al.*, “Bile acids PKA-dependently induce a switch of the IL-10/IL-12 ratio and reduce proinflammatory capability of human macrophages,” *J. Leukoc. Biol.*, vol. 94, no. 6, pp. 1253–1264, Dec. 2013, doi: 10.1189/jlb.0812396.

[72] M. Biagioli *et al.*, “The Bile Acid Receptor GPBAR1 Regulates the M1/M2 Phenotype of Intestinal Macrophages and Activation of GPBAR1 Rescues Mice from Murine Colitis,” *J. Immunol.*, vol. 199, no. 2, pp. 718–733, Jul. 2017, doi: 10.4049/jimmunol.1700183.

[73] L. Wang *et al.*, “Gut microbial bile acid metabolite skews macrophage polarization and contributes to high-fat diet-induced colonic inflammation,” *Gut Microbes*, vol. 12, no. 1, pp. 1–20, Nov. 2020, doi: 10.1080/19490976.2020.1819155.

[74] C. C. Bain *et al.*, “Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice,” *Nat Immunol*, vol. 15, no. 10, pp. 929–937, Aug. 2014, doi: 10.1038/ni.2967 http://www.nature.com/ni/journal/v15/n10/abs/ni.2967.html#supplementary-information.

[75] A. Rivollier, J. He, A. Kole, V. Valatas, and B. L. Kelsall, “Inflammation switches the differentiation program of Ly6C^{hi} monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon,” *J. Exp. Med.*, vol. 209, no. 1, p. 139, 2012, [Online]. Available: http://jem.rupress.org/content/209/1/139.abstract.

[76] B. Bernshtein *et al.*, “IL-23-producing IL-10Ra-deficient gut macrophages elicit an IL-22-driven proinflammatory epithelial cell response,” *Sci. Immunol*, vol. 4, p. 6571, 2019, Accessed: Jan. 07, 2020. [Online]. Available: http://immunology.sciencemag.org/.

[77] E. Larsson *et al.*, “Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88,” *Gut*, 2012, doi: 10.1136/gutjnl-2011-301104.

572 [78] A. M. Earley, C. L. Graves, and C. E. Shiau, “Critical Role for a Subset of Intestinal Macrophages in
573 Shaping Gut Microbiota in Adult Zebrafish,” *Cell Rep.*, vol. 25, no. 2, pp. 424–436, 2018, doi:
574 10.1016/j.celrep.2018.09.025.

575 [79] K. A. Earle *et al.*, “Quantitative Imaging of Gut Microbiota Spatial Organization,” *Cell Host Microbe*,
576 vol. 18, no. 4, pp. 478–488, 2015, doi: 10.1016/j.chom.2015.09.002.

577 [80] H. Seedorf *et al.*, “Bacteria from Diverse Habitats Colonize and Compete in the Mouse Gut,” *Cell*, vol.
578 159, pp. 253–266, 2014, doi: 10.1016/j.cell.2014.09.008.

579 [81] J. J. Faith *et al.*, “The Long-Term Stability of the Human Gut Microbiota,” *Science (80-.).*, vol. 341, no.
580 6141, p. 1237439, Jul. 2013, doi: 10.1126/science.1237439.

581 [82] D. Crespo-Piazuelo *et al.*, “Characterization of bacterial microbiota compositions along the intestinal
582 tract in pigs and their interactions and functions,” *Sci. Rep.*, vol. 8, no. 1, p. 12727, Dec. 2018, doi:
583 10.1038/s41598-018-30932-6.

584 [83] S. Gu, D. Chen, J.-N. Zhang, X. Lv, and K. Wang, “Bacterial Community Mapping of the Mouse
585 Gastrointestinal Tract,” *PLoS One*, vol. 8, no. 10, p. 74957, 2013, doi: 10.1371/journal.pone.0074957.

586 [84] G. He, R. A. Shankar, M. Chzhan, A. Samouilov, P. Kuppusamy, and J. L. Zweier, “Noninvasive
587 measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of
588 living mice with spatial and spectral EPR imaging,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 96, no. 8, pp.
589 4586–4591, Apr. 1999, doi: 10.1073/pnas.96.8.4586.

590 [85] L. Albenberg *et al.*, “Correlation between intraluminal oxygen gradient and radial partitioning of
591 intestinal microbiota,” *Gastroenterology*, vol. 147, no. 5, pp. 1055-1063.e8, Nov. 2014, doi:
592 10.1053/j.gastro.2014.07.020.

593 [86] I. I. Ivanov *et al.*, “Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria,” *Cell*, vol.
594 139, no. 3, pp. 485–498, 2009, doi: 10.1016/j.cell.2009.09.033.

[87] J. Bonnardel, C. Ment, D. Silva, J.-P. Gorvel, and H. L. Correspondence, “Innate and Adaptive Immune Functions of Peyer’s Patch Monocyte-Derived Cells,” *Cell Rep.*, vol. 11, pp. 770–784, 2015, doi: 10.1016/j.celrep.2015.03.067.

[88] C. A. Hickey *et al.*, “Colitogenic Bacteroides thetaiotaomicron antigens access host immune cells in a sulfatase-dependent manner via outer membrane vesicles,” *Cell Host Microbe*, vol. 17, no. 5, pp. 672–680, 2015, doi: 10.1016/j.chom.2015.04.002.

[89] K. R. James *et al.*, “Distinct microbial and immune niches of the human colon,” *Nat. Immunol.*, vol. 21, no. 3, pp. 343–353, Feb. 2020, doi: 10.1038/s41590-020-0602-z.

[90] J. L. M. Welch *et al.*, “Spatial organization of a model 15-member human gut microbiota established in gnotobiotic mice,” *PNAS*, 2017, doi: 10.1073/pnas.1711596114.

[91] S. M. Parigi *et al.*, “The spatial transcriptomic landscape of the healing intestine following damage,” *bioRxiv*, 2021, doi: 10.1101/2021.07.01.450768.

[92] J. Garate *et al.*, “Imaging mass spectrometry increased resolution using 2-mercaptobenzothiazole and 2,5-diaminonaphtalene matrices: application to lipid distribution in human colon,” *Anal. Bioanal. Chem.*, vol. 407, no. 16, pp. 4697–4708, 2015, doi: 10.1007/s00216-015-8673-7.

[93] M. E. V. Johansson, J. M. H. Larsson, and G. C. Hansson, “The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host–microbial interactions,” *Proc. Natl. Acad. Sci.*, vol. 108, no. Supplement 1, pp. 4659–4665, Mar. 2011, doi: 10.1073/PNAS.1006451107.

[94] C. Tropini, K. A. Earle, K. C. Huang, and J. L. Sonnenburg, “The Gut Microbiome: Connecting Spatial Organization to Function,” *Cell Host Microbe*, vol. 21, 2017, doi: 10.1016/j.chom.2017.03.010.

[95] T. M. Fenton, P. B. Jørgensen, O. H. Nielsen, B. Johansson-Lindbom, and W. W. Agace, “Immune Profiling of Human Gut-Associated Lymphoid Tissue Identifies a Role for Isolated Lymphoid Follicles

in Priming of Region-Specific Immunity," *Immunity*, vol. 52, pp. 557–570, 2020, doi: 10.1016/j.immuni.2020.02.001.

[96] N. A. Scott and E. R. Mann, "Regulation of mononuclear phagocyte function by the microbiota at mucosal sites," *Immunology*, vol. 159, no. 1, pp. 26–38, Jan. 2020, doi: 10.1111/imm.13155.

[97] I. Gabanyi, P. A. Muller, L. Feighery, T. Y. Oliveira, F. A. Costa-Pinto, and D. Mucida, "Neuro-immune Interactions Drive Tissue Programming in Intestinal Macrophages," *Cell*, vol. 164, no. 3, pp. 378–391, Jan. 2016, doi: 10.1016/J.CELL.2015.12.023.

[98] A. Sehgal, D. S. Donaldson, C. Pridans, K. A. Sauter, D. A. Hume, and N. A. Mabbott, "The role of CSF1R-dependent macrophages in control of the intestinal stem-cell niche," *Nat. Commun.*, vol. 9, no. 1, pp. 1–17, 2018, doi: 10.1038/s41467-018-03638-6.

[99] K. Asano *et al.*, "Intestinal CD169+ macrophages initiate mucosal inflammation by secreting CCL8 that recruits inflammatory monocytes," *Nat. Commun.*, vol. 6, no. 1, p. 7802, Nov. 2015, doi: 10.1038/ncomms8802.

Box 1: Cross-sectional macrophage localization in mice

Different subsets of macrophages appear to co-exist in the intestine. $CD206^+$ macrophages are considered anergic despite their bactericidal activity, as they do not normally elicit an inflammatory response [20]. Across the depths of the intestine, LP and muscularis mucosae macrophages show unique tissue adaptions and gene expression profiles [97]. Further research in the neuro-immune interactions revealed that muscularis macrophages express a tissue-protective transcriptome which is imprinted by β2 adrenergic receptor signaling [97]. Two subsets of macrophages occupying distinct niches across the depths of the colonic lamina propria were identified in a recent scRNA-seq study [35]. $CD206^+$ macrophages are localized around the base of the crypt and have an anti-inflammatory transcriptome whereas pro-inflammatory $CD11c^+$ macrophages were localized close to the luminal surface [35]. This was confirmed in another scRNA-seq study profiling macrophages and DCs in the colon of specific pathogen-free (SPF) and germ-free (GF) mice identified $CD206^+$

642 CD169^{+} macrophages and CD121b^{+} CD11c^{+} macrophages to be affected by microbiota colonisation [36].
643 Comparing steady state and peak of inflammation in a murine colitis model revealed a TF-dependent accu-
644 mulation of pro-inflammatory CD11c^{+} macrophages at peak of inflammation, reinforcing the importance of
645 TFs in mediating both function and potentially localization of macrophages. Macrophages at the base of
646 colonic crypts were previously found to be essential for maintenance of the epithelial stem cell niche and
647 regulation of Paneth cell function [16], [98], [99]. Following mucosal injury with DSS, these macrophages
648 mediate influx of monocytes in a CCL8-dependent manner [99].

649

650

651