A Cross-Talk Between Microbiota-Derived Short-Chain Fatty Acids and the Host Mucosal Immune System Regulates Intestinal Homeostasis and Inflammatory Bowel Disease

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A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease.

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ABSTRACT

Gut microbiota has a fundamental role in the energy homeostasis of the host and is essential for proper “education” of the immune system. Intestinal microbial communities are able to ferment dietary fiber releasing short-chain fatty acids (SCFAs). SCFAs, particularly butyrate (BT), regulate innate and adaptive immune cell generation, trafficking, and function. For example, BT has an anti-inflammatory effect by inhibiting the recruitment, and proinflammatory activity of neutrophils, macrophages, dendritic cells and effector T cells, and by increasing the number and activity of regulatory T cells. Gut microbial dysbiosis, i.e. a microbial community imbalance, has been suggested to play a role in the development of inflammatory bowel disease (IBD). The relationship between dysbiosis and IBD has been difficult to prove, especially in humans, and is probably complex and dynamic, rather than one of a simple cause and effect relationship. However, IBD patients have dysbiosis with reduced numbers of SCFAs-producing bacteria and reduced BT concentration which is linked to a marked increase in the number of proinflammatory immune cells in the gut mucosa of these patients. Thus, microbial dysbiosis and reduced BT concentration may be a factor in the emergence and severity of IBD. Understanding the relationship between microbial dysbiosis and reduced BT concentration to IBD may lead to novel therapeutic interventions.

Keywords: microbiota, immune system, short chain fatty acids, butyrate, inflammation, inflammatory bowel disease.
HOST COLONIZATION BY MICROBIOTA

The body of mammals is normally colonized by bacteria, fungi, and viruses, collectively referred as the commensal microbiota. In humans, although distinct microbiota inhabit all body surfaces exposed to the environment, the largest ($10^{14}$ microbes) and most varied microbiota population ($100$-$1000$ species) resides in the gut (1). Microbial density increases along the gut, with few bacteria in the upper small gastrointestinal tract until it reaches its peak in the colon, which contains the highest microbial diversity and load (up to $10^{12}$ bacteria/gram of faeces) (2).

Despite considerable individual variation (3), $\approx 90\%$ of adult gut microbiota belong to major bacterial phyla gram-negative *Bacteroidetes* (*Bacteroides, Prevotella*, and *Porphyromonas*) and gram-positive *Firmicutes* (*Clostridia* and *Bacilli*) (4). The highly stable and shared component of microbiota between individuals is known as “core microbiota”, as opposed to a highly variable component that is capable of fluctuate very fast in response to external stimuli and to adapt to new conditions (5).

Microbiota form a symbiotic relationship with their host: microbes benefit from the nutrient-rich environment of the gut, and on the other hand, microbiota encode hundreds of genes that are absent in the human genome and modulate several biological functions of the host, including nutrient processing, maintenance of energy homeostasis and immune system development (6). Despite bacterial transmission from the mother to the fetus can occur during human pregnancies (7), the greatest microbiota colonization is initiated by maternally derived microbes at birth (8). The delivery mode is a major determinant of the newborns microbiota composition, babies delivered by cesarean section (C-section) acquire a microbiota that is enriched in mother’s skin microbes while vaginally delivered babies acquire a microbiota resembling those of the maternal vagina (9). The microbiota colonization during the first weeks of newborn life plays a essential role in the development and education of the host mammalian immune system (10) and babies delivered by C-section has been associated with increased risk
for development of obesity, asthma, allergies and immune deficiencies (11). However, the composition of microbiota community is rapidly evolving during the first year of life, when Bifidobacterium is dominant, microbial diversity gradually increases, reaching an adult stable configuration at age of 2-3 years (4). During this evolution the host genetics can shape the microbiome composition (12), but the environmental factors (e.g. dietary and antibiotic use) have a major impact in shaping this community (4, 8). The adult microbiota composition of C-section delivered babies is comparable to vaginally delivered (3, 13), suggesting that delivery mode does not affect adult microbiota composition. Dietary and microbial antigens can also be absorbed in the mucosa, transferred across the placenta from the mother blood to the fetus and affect the development of fetal immune system. For example, maternal consumption of vitamin A and subsequent generation of retinoic acid signalling is important for modulation of fetal lymphoid tissue inducer (LTi) cell differentiation which impacts on the size of secondary lymphoid organs and the efficiency of immune responses in the adult offspring (14). Metabolites produced by microbiota during late pregnancy protect also against the development of allergic responses in adult offspring (15). Recently, a study in pregnant mice colonized with the bacteria that then returned to a germ-free (GF) state before giving birth, showed that microbiota metabolites produced in pregnancy leads to increased numbers of group 3 innate lymphoid cells (ILC3s) and intestinal mononuclear cells in gut of newborns (16). This increase persisted until at least 8 weeks after birth, suggesting that temporary microbiota colonization has long-term effects on the immune system. Maternally acquired antibodies during pregnancy specific from commensal microbes can also regulate T helper (Th) cell activation in gut of neonatal mice (17). Therefore, the exposure to various microbial antigens, metabolites and antibodies in the fetus contributes to the early development of a functional immune system (7). These studies suggests that both prenatal (maternal) and the early life (infant) microbiota have crucial roles in the development of immune system.
Decades of studies in GF animals showed that multiple microbial species have an important role in the development of gut-associated lymphoid tissue (18-20) and in the colonization of the gut wall by immune cells, such as the so-called intraepithelial lymphocytes (21-24), lamina propria neutrophils (25), dendritic cells (26), ILC3s (16, 27, 28), mucosal-associated invariant T (MAIT) cells (29, 30), TCR αβ Th17 cells and TCR γδ IL-17-producing cells (31-33), regulatory T cells (Tregs) (34), and immunoglobulin (Ig)A secreting plasma cells (35, 36). Moreover, the maturation of specific immune cells depends on certain host-specific bacterial species (26). For example, particular bacteria have been linked to the development of Th17 cells (37, 38) and others are involved in the expansion of Foxp+ Tregs (34, 39). Despite these studies in mice there are no studies about the effect of microbiota in human cellular subset composition (40). However, human healthy individuals have high level of interindividual variation in immune cell subset composition and the largest influence on immunological variation identified was cohabitation, with 50% less immune cell variation between individuals who share the same environment (41). Microbiota composition may have a role, for example cohabiting individuals show convergence in microbiomes (42, 43) possibly via direct transmission (44). Microbiota protect also the host from opportunistic pathogenic infection (45, 46), since they inhibit the adhesion to the gut mucus and colonization by pathogens (47). In addition to this barrier effect, commensal microbiota produce bacteriocins and short chain fatty acids (SCFAs), compounds that are able to inhibit the growth of potentially invading pathogenic bacteria (47, 48). Louis Pasteur two centuries ago already said that “the role of the infinitely small in nature is infinitely great”.

MICROBIOTA AND THE GUT IMMUNE SYSTEM

Gut-associated lymphoid tissue (GALT), together with intestinal draining lymph nodes (LN), serve as the major sites of adaptive immune cell priming. Antigens sampled through M
cells are taken up by antigen-presenting cells for processing and presentation to lymphocytes in gut-associated lymphoid tissue. In addition, some bacteria can directly colonize gut-associated lymphoid tissue (i.e. lymphoid follicles, Peyer’s patches and the mesenteric lymph nodes) in healthy mammals. (49-51).

The majority of adaptive immune cells in the epithelium are intraepithelial lymphocytes (IELs) (52). These cells are located between intestinal epithelial cells (IECs) in a ratio of approximately one lymphocyte for every six to ten IECs (53). For example, small intestine harbor ≈ 50 millions intraepithelial T lymphocytes (IELs) representing up to half the number of T cells in the mice (54). IELs include conventional CD8+ and CD4+ αβ T cells, as well as unconventional T cell types including TCRγδ+ and CD8αα+ TCRαβ+ lymphocytes (53). TCRγδ+ constitute a major proportion of the IELs population that contributes to the maintenance of the epithelial barrier and gut homeostasis (53). Conventional TCRαβ+ IELs derive from naïve T cells that have undergone activation in GALT or LNs and subsequently homed to the intestine to generate epithelial resident effector memory T cell population. Microbiota diversity induces a progressive and considerable increase in the numbers of both CD8αβ+ and CD8αα+ TCRαβ+ IELs (26, 55, 56). The absence/reduction of CD8αβ+ TCRαβ+ IELs in GF mice (21-24) supports a major role of the microbiota in the generation of these cells (22, 57, 58). Underlying the intestinal epithelium is the lamina propria. The most abundant immune population in the lamina propria is macrophages, which rapidly phagocytize microbes that breach the gut physical barrier. These macrophages predominantly represent tissue-resident non-migratory macrophages, belonging to the functional M2 subtype characterized by the production of large amounts of IL-10. Besides macrophages, lamina propria is also rich in dendritic cells. These cells can extend dendrites between IECs without disrupting the intestinal barrier function, acquire antigens from microbiota and subsequently migrate to mesenteric lymph nodes, where they are presented these antigens to T cells. In normal conditions, dendritic cells induce
tolerance to commensal microbiota antigens, through the secretion of TGF-β and retinoic acid induces de differentiation of naïve CD4+ T cells in Tregs (59).

Part of the adaptive immune system, B cells are one of the predominant population in the lamina propria of small intestine and colon (60). Was estimated that 80% of human and mouse fully differentiated B cells (plasma cells) are located in the intestinal lamina propria. One of the most prominent effects of microbiota colonization is the induction of secretory IgA (35, 36). The switch of IgM-positive B cells to IgA-positive B cells occur mostly in GALT, a T dependent and independent pathway, induce the production of IgA that is next secreted at the epithelial level. IgA binds to surface molecules expressed by microbes causing their agglutination in the gut lumen (61). High-affinity antigen-specific IgA, produced by a T dependent pathway, binds to surface molecules expressed by pathogenic bacteria and their toxins and helps in their neutralization (62-64). On the other hand, commensal bacteria might also become coated with IgA but the level of these process is generally been believed to be lower as compared to pathogenic bacteria (65). This is probably due to the fact that commensal microbiota induces low-affinity IgA in a T cell independent manner, since mice lacking cognate T-cell-B-cell interactions or mice lacking T cells still mount IgA responses to commensal (62-64).

Lamina propria is also rich in conventional αβ T cells, ≈ 15% of CD8+ and ≈ 85% of CD4+ T cells in mice (38). CD4+ T cells in the lamina propria of small intestine and colon are predominantly RORγt+ Th17 cells and RORγt+ Foxp3+ Tregs (66). RORγt+ Th17 represents 40% of CD4+ T cells in the small intestinal (38) and about 50% in the colonic lamina propria (67). Tregs represents 20% of CD4+ T cells in the small intestinal lamina propria and about 30% in the colonic lamina propria (68). Microbiota colonization is a prerequisite to gut accumulation of both RORγt+ Th17 cells (31, 67, 69) and Foxp3+ Tregs (34, 70, 71). Microbiota induces antigen-specific RORγt+ Th17 cells responses in the gut (72), and these cells are also
essential for protection against opportunistic infections. On the other hand, Tregs suppress a wide range of immune cells, maintain tolerance to environmental antigens and avoid the risk of gut inflammation (73). These cells are characterized by the permanent expression of high levels of the transcription factor Foxp3, which is considered to confer their suppressive phenotype (74). The majority of Foxp3+ Tregs develop in the thymus - thymus-derived Tregs (tTreg) -, alternatively naïve CD4+ T cells upon antigen exposure and in the presence of TGF-β and retinoic acid can also be induced to express Foxp3, producing peripherally-induced Tregs (pTregs) in extrathymic tissues (75). Some studies suggest that a substantial proportion of the colonic Tregs arises extrathymically from antigen-specific interactions with the colonic microbiota (34, 70, 71). These pTregs express a unique subset of TCRs (which do not overlap with the tTreg TCR repertoire) and recognized epitopes derived from commensal microbiota (70, 71).

Innate lymphoid cells (ILCs) are resident innate cells in the lamina propria that share functional characteristics with differentiated T cells. ILCs can be subdivided into three different groups referred to as group 1, 2, and 3. Group 3 ILCs in mice is further categorized in CCR6+ LTi cells and CCR6- ILC3s cells, some CCR6- ILC3 cells express a natural cytotoxicity-triggering receptor (NCR, NKp46) and others not. At the present time, ILC3s appears to be the most dependent on the presence of microbes. Microbiota induces CCR6+ LTi cells that promote the formation of secondary lymphoid organs (18-20) and NCR+ ILC3s numbers (16, 27, 28). Both CCR6+ ILC3 cells and NCR+ ILC3 cells are major producers of IL-22 and IL-17A, cytokines that sustain epithelial integrity and regulate mucosal immunity (76). Microbiota colonization stimulates IL-22 production by ILC3s (16, 27, 28). IL-22 maintains the intestinal barrier function by promoting the production of antimicrobial peptides and mucus involved in antimicrobial host defense, and promote wound healing, tissue remodeling and repair (28, 77, 78). IL-22 induces also fucosylated carbohydrate synthesis in IECs (79) and these
carbohydrates can supplies a selective advantage to beneficial commensal bacteria that can metabolize these compounds (79, 80). On the other hand, IL-17 is essential for regulating mucosal host defense against invading pathogens, and the disruption of this signaling in the gut epithelium results in microbiota dysbiosis (81). ILC3s have other important functions, namely MHCII+ ILC3s reduce commensal-specific Th responses by the antigens presentation and induction of a CD4+ T cells negative selection process (82). In addition, ILC3-mediated release of granulocyte/macrophage colony-stimulating factor via IL-1β maintains colonic Tregs homeostasis (83). Furthermore, ILC3-derived lymphotoxins orchestrate IgA secretion and control microbial composition in the gut (84). All of these properties makes that ILC3s depletion promotes the dissemination of bacteria in gut and drive systemic inflammation (28, 49, 85).

MICROBIOTA AND SHORT CHAIN FATTY ACIDS

The innate immune cells and non-immune cells of the host receive direct stimulus from the microbial antigens through microbe-associated molecular patterns (MAMPs), such as toll-like membrane receptors (TLRs) and intracellular nucleotide-binding oligomerization domain (NODs). The continuous intestinal absorption of low-level microbial products such as NODs and TLRs ligands, microbial antigens into the systemic circulation represents a crucial aspect of the cross-talk between the microbiota and the systemic immune system (45). For example, bone marrow progenitor cells can directly sense microbiota signals (e.g. LPS) via TLRs (86) and microbiota can control immunity through regulation of hematopoiesis at primary immune sites (87). Indeed, GF mice display reduced proportions and differentiation potential of specific myeloid progenitors cells, have decrease resident myeloid cell populations in bone marrow and reduction of in monocytes, macrophages and neutrophils in peripheral sites (87). On the other hand, microbiota colonization can enhance myelopoiesis and increase the myeloid cells in
peripheral sites (87). Microbiota can also affect TCRαβ+ CD8+ and CD4+ T cells, TCRγδ+ and ILCs in other peripheral sites (e.g. lymph nodes, lung, skin and brain) (88, 89) and regulate immune responses in peripheral sites (e.g. in pancreas (90) and brain (89)).

Commensal microbial metabolism produces essential vitamins (e.g. group B and K vitamins), SCFAs as well metabolizes compounds (e.g. bile acids, sterols and xenobiotics) that are transformed into secondary bioactive metabolites and contribute to the gut “metabolome” (4, 91). Besides structural components of bacteria, many of metabolites derived from commensal microbial metabolism have been implicated in the development and function of immune cells, e.g. vitamin B-based metabolites in MAIT cells (30, 92) and sphingolipids in invariant Natural Killer T (iNKT) cells (93). However, the best-studied microbial metabolites that influences immune system homeostasis are SCFAs, in particular acetate, propionate and butyrate (BT) (Figure 1). Moreover, the most abundant commensal bacteria in gut, *Bacteroidetes* and *Firmicutes*, are able to ferment non-digestible carbohydrates releasing SCFAs (94) that can be found at high concentrations in healthy gut (e.g. 20 mM BT in colonic lumen) (95). Among SCFAs, BT has multiple regulatory roles at gut level, exerting an anti-inflammatory effect on both intestinal epithelial cells and immune cells (96, 97). This article highlights our current understanding of how SCFAs, specifically BT, interact with immune cell generation, trafficking, and function, and how these interactions influence host gut health (Figure 1). SCFAs produced by the microbiota can also translocate from the gut to the bloodstream and regulate host systemic immune system (88). For example, SCFAs can inhibit hematopoiesis and inducing accumulation of immature dendritic cells in bone marrow (87, 98), can increase Tregs in lungs (15) and protect against the development of lung allergic inflammation (15, 98, 99). SCFAs are also key molecules that modulate microglia (the brain’s macrophages) maturation, morphology and function (100) and can attenuate neuroinflammation (101).
SCFA CROSSTALK WITH THE INTESTINAL EPITHELIUM

The intestinal barrier is a functional entity separating the gut lumen (and microbiota) from the host. Several elements participate in this barrier including a single monolayer of polarized IECs, represented by absorptive enterocytes, mucus-secreting goblet cells, hormone-producing enteroendocrine cells, and antimicrobial peptides and lectins-secreting Paneth cells (102). IECs secrete mucus and antimicrobial proteins (e.g. C-type lectin RegIII-β and RegIII-γ) and BT increase the expression of mucins (103) and anti-microbial peptides (104, 105) in IECs. Interestingly enough, BT leads to IECs inflammasome activation and production of anti-inflammatory IL-18 (106, 107), a cytokine involved in mucins and anti-microbial peptide production and in the control of gut microbiota composition (108). Additionally, BT promotes intestinal barrier function due to its ability to suppresses intestinal stem cell proliferation (109), promote crypt IECs differentiation and modulation of the expression of tight junction proteins (110, 111). IECs modulate also IgA transectosis to gut lumen that when bound to specific bacteria causes their agglutination and anchorage to the mucus, preventing their direct contact with the epithelium (102). SCFAs added to parenteral nutrition increases secretory IgA levels in the intestinal mucosa of mice (112). It is known that IECs supports IgA class switching (113). BT induces the expression of TGF-β in IECs (39), and TGF-β indirectly induces B cell class switching to produce IgA (64). In addition, SCFAs regulate directly the metabolism and gene expression in B cells and promotes the secretion of IgA (114, 115). This data suggests that BT induction of secretory IgA is a T cell independent mechanism to maintain control the commensal microbiota composition (115, 116).

IECs not only physically separate the luminal contents from the internal milieu but also mediate the cross-talk between the microbiota and the immune cells in the lamina propria (117). This communication is mediated via cell-cell contact (with T cells and dendritic cells) and
chemokine and cytokine signaling. IECs can produce some cytokines (e.g. IL-10 and TGF-β) that affect immune cells (118-120) and respond to cytokines and other mediators produced by immune cells in the lamina propria (117) (Figure 1). IECs receive direct stimulus from the microbial antigens through (TLR)1-5 and 9 and NOD2 (121). The activation of these receptors induces the production of proinflammatory cytokines (122) and chemotactic factors for both myeloid and lymphoid cells (123). However, IECs express low levels of TLRs on the apical side (pole facing the intestinal lumen) and higher levels on the basolateral side (that communicates with lamina propria), being essentially stimulated by invasive bacteria (121).

Interestingly, BT decreases TLR4 expression and production of proinflammatory cytokines in IECs (124, 125). On the other hand, proinflammatory cytokines can drive the expression of neutrophil chemoattractant chemokines in IECs (126), such as IL-8, a chemokine that BT can significant decrease the secretion (124, 127). BT was also shown to inhibit the expression of the chemokines CXCL5 and CXCL11 (128). In conclusion, BT has a direct anti-inflammatory effect on IECs and in the crosstalk between IECs and gut immune cells.

**SCFA CROSSTALK WITH THE IMMUNE SYSTEM**

Understanding the complex molecular mechanisms underlying the anti-inflammatory effect of SCFAs, specifically BT, has been difficult because they interact with multiple signaling molecules. First, BT was demonstrated to be a physiologic ligand of membrane G-protein-coupled receptors GPR109A (129), GPR41 and GPR43 (130-132). GPR109A are not activated by propionate and acetate. However, acetate can activate GPR43 and propionate both GPR41 and GPR43, although with different affinities (129-132). GPR109A is a receptor expressed by some immune cells (e.g. macrophages, dendritic cells and neutrophils) and the activation of this receptor decrease the production proinflammatory mediators (133, 134). GPR41 and GPR43 are also expressed by some immune cells such as monocytes, dendritic
cells, eosinophils and neutrophils (130, 131, 135-138). At least GPR43 seems to be important in the regulation of immune responses, since mice lacking GPR43 develop exacerbated inflammation in models for colitis due to higher production of inflammatory mediators and increased recruitment of immune cells (135, 136). SCFAs uptake inside the immune cells is dependent on monocarboxylate transporters (MCTs) (139). Secondly, intracellular BT (and propionate but not acetate) control gene expression by direct inhibition of histone deacetylases (HDACs) (140-144). BT inhibits the activity of most zinc-dependent HDACs, except class IIa HDACs 6 and 10 isoforms, (140-144) inducing the hyperacetylation of histones, resulting in open structure of chromatin and leading DNA accessible to initiate transcription of genes. BT acting has a histone deacetylases inhibitor (HDACi) can overcome the inflammatory response by inhibition of nuclear factor kappaB (NF-kB) activation and production proinflammatory mediators (97, 145). Synthetic HDACi are being developed for treatment of proinflammatory diseases such as inflammatory bowel disease (IBD) (146). BT can also induce the expression of nuclear receptor peroxisome proliferator activated receptor (PPAR)γ (147), which exerts anti-inflammatory effects through antagonism of NF-kB (148). However, BT can induce other epigenetic modifications, namely hyperacetylation of nonhistone proteins (149), selective inhibition of histone phosphorylation (150), histone methylation (151) and DNA methylation (152). Third, metabolism has a direct role in regulating immune cell function. BT can be metabolized in tricarboxylic acid cycle, inhibiting glycolysis (153) and inducing autophagy (154). BT increase Akt/mTOR activity a pathway implicated in the regulation of immune responses (155). Therefore, the integration of BT into cellular metabolism can regulate cell energy status and several signaling processes (156, 157).

**Immune cell differentiation and recruitment**
Lymphoid and myeloid cells develop largely from hematopoietic stem cells resident in bone marrow. As previously discussed, SCFAs can inhibit hematopoiesis (87, 98), particularly myelopoiesis and decrease resident mature myeloid cell populations in bone marrow (15, 98). Consistent with these data, *in vitro*, BT decreases the differentiation of monocyte to derived macrophages (158) and dendritic cells (159-161). BT impairs the differentiation and maturation of dendritic cells by its HDACi activity (162, 163). On the other hand, histone modifications are essential for B cell development and proliferation (164), BT acting at this level can modulate B cell differentiation and induce B cell accumulation (165, 166). In addition, BT can affect the recruitment of immune cells to the periphery. Monocyte chemoattractant protein-1 (MCP-1) is one of the key chemokines that regulate migration and infiltration of macrophages into the gut, BT reduces the release of MCP1 and vascular cell adhesion molecule-1 (VCAM1) (167, 168), decreasing the recruitment and migration of macrophages (168, 169). Macrophages and dendritic cells can induce the recruitment of leukocyte (chemotaxis) (170). BT reduce the release of several proinflammatory chemokines from human monocyte-derived dendritic cells (e.g. CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11) (171) and IECs (e.g. CXCL5 and CXCL11) (128) and can modulate leukocyte trafficking. BT reduces surface expression of chemoattractant receptors C5aR and CXCR2 in neutrophils and inhibits their chemotaxis *in vivo* acting through GPR43 receptor (136). Therefore, BT decreases neutrophils recruitment *in vivo* to the gut (125, 172). BT treatment reduces also the number of neutrophils in crypt and surface epithelia of IBD patients (145). Interestingly enough, BT reduces the recruitment of circulating T cells to the inflammatory sites (136, 173). The surface expression of chemoattractant receptors CXCR2 in lymphocytes is reduced by BT treatment, contributing to a decrease in T cells migration to inflammatory sites (136). On the other hand, compounds with HDACi activity stimulates thymic production of tTregs and increases their peripheral numbers in normal mice (174). BT can also increase tTregs numbers in colon (138). The oral
administration of SCFAs can mediate the migration of tTregs into the gut through the upregulation of GPR15 receptor, a receptor implicated in the homing of mice tTregs in colon (138, 175, 176). GPR43 and GPR41 receptors are also expressed in leukocytes (130, 137). SCFAs induced lung Tregs suppressor function by a GPR41-dependent manner (177), suggesting that these receptors may have a chemoattractant effect for Tregs.

Decrease in proinflammatory mediators

Macrophages through the secretion of cytokines can control the inflammatory state of the gut (178). The activation and production of proinflammatory cytokines by macrophages is regulated by histone modifications (179, 180). Therefore, BT acting as a HDACi, inhibits macrophages-mediated production of proinflammatory mediators (e.g. TNF-α, IL-6) *in vitro* (181, 182) and *in vivo* (94, 181). In addition, BT induces their apoptosis (183) decreasing the number of proinflammatory M1 macrophages *in vivo* (145, 181). Dendritic cells are also able to drive inflammatory T cell responses in gut. However, BT decreases dendritic cell co-stimulatory molecules (184, 185), decreases their activation (107) and suppresses the production of Th1 cytokines (e.g. TNF-α, IL-1β, IL-8) (180, 184, 186). Neutrophils can also release multiple proinflammatory mediators (187). BT reduces the production of proinflammatory cytokines (e.g. TNF-α and cytokine-induced neutrophil chemoattractant-2) in neutrophils (136, 173, 188), decreases their phagocytosis capacity and cytotoxic effect (189-193). BT can induce apoptosis (194) and the induction of apoptosis might be involved the observed decrease of neutrophil numbers *in vivo* (195). Mast cells are also traditionally associated with inflammation. However, BT through HDACi activity has been shown to inhibits cell proliferation, increases cell apoptosis and suppress mast cell proinflammatory cytokines production (196).
The activation of naïve T cells requires presentation of antigens to T cells by antigen presenting cells and, as described above, BT modifies the biology of antigen presenting cells. Therefore, BT inhibits T cell activation (197) in response to antigens (197, 198) and mitogens (199). Naïve T cells begin proliferating, before their acquisition of effector functions. However, BT acting as a HDACi blocks cell cycle in G1 phase, inhibiting T cells cell-cycle progression (184, 200-202) and the proliferation of both CD4+ and CD8+ T cells (96, 160, 203). On the other hand, upon activation Th1, Th2 and Th17 cells use primarily glycolysis for energy generation (204). Lactate anions produced during glycolysis are exported out of the effector T cells by monocarboxylate transporters (MCTs) (205). MCTs are bidirectional transporters and extracellular BT, by competing with intracellular lactate for MCTs, can lead to inhibition of lactate efflux, induce intracellular accumulation of lactate, feedback inhibition of glycolysis (153). Therefore, the integration of BT in T cell metabolism inhibits glycolysis (153) and Th cell differentiation and proliferation (206). BT can also induce apoptosis of CD4+ and CD8+ effector T cells (207, 208). BT, in naïve CD4+ T cells, affects neither the acetylation nor the expression levels of “masters” transcription factors T-bet, Gata3, and RORγt for the development of Th1, Th2 and Th17, respectively (209). However, BT inhibited transducer and activator of transcription (STAT)1 (210, 211) and STAT5 (212) signaling pathways in activated CD4+ T cells and inhibits the production of Th1 (197, 198, 213, 214) and Th17 cytokines (163, 215). These data suggest that BT can inhibit the activation and production of proinflammatory cytokines.

Increase in anti-inflammatory mediators

Commensal microbiota, particularly the Firmicutes (Clostridium clusters IV and XIVa) and Bacteroides fragilis are potent inducers of pTregs differentiation (34, 39). These bacteria are also major producers of SCFAs (34, 39). SCFAs, specifically BT, augmented directly the

Interestingly enough, other SCFAs are also able to induce Foxp3+ expression, promoting Tregs accumulation in lungs and inhibiting the development of allergic responses (15). Recently, it has also been shown Clostridium species produce SCFAs from butyrylated high-amylase diet and induced specifically RORγt+ Tregs in mouse colon lamina propria (67). However, in another study, SCFAs was quantified by liquid chromatography-mass spectrometry (LC-MS) in cecal content and they did not found correlation between SCFAs levels in gut and RORγt+ Treg frequency (69), most probably because SCFAs are efficiently and quickly absorbed from gut lumen to IECs (110). Butyrylated starches are chemically modified starches, in which the acyl group is linked to the starch framework by an ester bond, found to be used by colonic bacteria that deliver esterified BT and propionate that leads to changes in gut microbiota composition. Therefore, normal dietary resistant and butyrylated starches can have different effects on microbiota profiles and in the induction of RORγt+ Tregs. The induced RORγt+ pTregs have high levels of IL-10 production, a stable regulatory function even in inflammatory conditions, (219) and can regulate Th1, Th2 and Th17 cell responses (69, 220). RORγt+ Tregs are mostly negative for neuropilin-1 (Nrp1) and Helios expression and are not present in GF conditions (67, 69, 219). Another question arising from these studies is whether the Foxp3+ Tregs induced by BT are antigen-specific. BT promotes the differentiation of colonic OVA-specific OT-II Foxp3+ T cells following the adoptive transfer of naïve OT-II CD4+ T cells and oral administration of OVA antigen (107, 209). On the other hand, the administration of BT failed to increase colonic Tregs in GF mice, suggesting that for the induction of pTregs naïve CD4+ cells need to recognize bacterial antigens (67, 69, 209). Therefore, Foxp3 expression needs to synergize with TCR signals to the generation of functional pTregs (221) and BT only “burst” the conversion and expansion of antigen-specific Foxp3+ pTregs.
BT facilitates pTregs differentiation by direct and indirect mechanisms. First, BT acting directly in naïve CD4+ T cells as a HDACi enhance acetylation status of histone H3 in the promoter and CNS3 enhancer regions of the Foxp3 gene loci leading to expression of Foxp3 protein (94, 209). Compounds with HDACi activity increase Foxp3 expression and enhance the suppressive function of Tregs (222, 223). Second, TCR activation and induction of anergy in peripheral CD4+ T cells, a state where the cell lose the ability to proliferate in response to antigens, can gave rise to pTregs (224). In addition, inhibition of glycolysis promotes Tregs differentiation (225). Therefore, BT induces anergy (197, 198, 213, 214) and blocks glycolysis (153), promoting the generation of pTregs (225). BT can indirectly induce pTregs by acting in dendritic cells and B cells (59). A study showed that the treatment of dendritic cells in vitro with BT markedly enhanced the ability of dendritic cells to induce Foxp3 expression in naïve CD4+ T cells (94). Another study showed that BT induces Raldh1 expression and promotes retinoic acid production in dendritic cells (107). Lamina propria plasma cells can also produce IL-10 (226), TGF-β and retinoic acid that induce Foxp3+ Tregs (227). BT switches the differentiation of B cells into plasma cells to IL-10+ producing B cells (Breg) (228, 229). Therefore, BT regulate the capacity of B cells to produce IL-10 and induce pTregs (230).

The anti-inflammatory cytokines, IL-10 and TGF-β, promote a microenvironment that can modulate the function of other gut immune cells. For example, CD4+Foxp3+IL10+ (Tr1) cells are present in high numbers in the intraepithelial and lamina propria of small intestine (231). These cells are induced by chronic activation of CD4+ T cells by antigen in the presence IL-10. However, BT treatment can induce directly by HDACi activity the development of Tr1 cells (107, 155). BT promotes also the increase in anti-inflammatory M2 macrophages (232-234) acting directly in these cells as a HDACi (234) and indirectly by the induction of pTregs (235) and production of Th2 cytokines, such as IL-10 (138, 188) and IL-5 (236). The anti-inflammatory microenvironment can inhibit the expression of costimulatory molecules, MHCII
levels and proinflammatory cytokine production in dendritic cells, inducing tolerogenic dendritic cells (237). Recently, it has been shown that SCFAs stimulate intestinal epithelial cells to induce mucosal tolerogenic CD103+ dendritic cells (238). On the other hand, tolerogenic dendritic cells can induce Foxp3+ Tregs, Tr1 cells and Bregs cells that produce the immunosuppressive cytokines (237). ILC3s play also a key role in regulation of gut homeostasis. BT promotes the production of retinoic acid in IECs (239) and dendritic cells (107) that is important for modulation of ILC3s differentiation (14). On the other hand induces the production of TGF-β (39) that control ILCs plasticity (240, 241). Unpublished data suggest BT as a modulator of ILCs and that the regulation of ILCs by BT was associated with maintaining gut homeostasis (242).

In conclusion, the adult gut immune system may tolerate self and harmless non-self antigens such as commensal microbiota, while maintaining the ability to recognize and induce a robust proinflammatory response against pathogenic bacteria. Understanding how the gut immune system discriminates between commensals and pathogens is a major challenge, because both express similar microbe-associated molecular patterns. The most abundant commensal bacteria in gut (Bacteroidetes and Firmicutes) produce SCFAs such BT. BT increase regulatory Gata3+ Tregs (243, 244), RORγt+ Tregs (67), Tr1 cells (107, 155), Bregs (228, 229), M2 macrophages (232-234), tolerogenic dendritic cells (237), and decreases proinflammatory mediators. These findings elucidate pathways through which BT plays an active role in modulating the mucosal immune system to establish a “tolerant” phenotype against beneficial commensal microbiota (Figure 1). Thus, high levels of BT tends to limit immune responses towards the commensals and contribute to the maintenance of microbiota diversity. Upon infection by specific pathogenic bacteria, there is a substantial remodelling of commensal microbiota, resulting in low concentration of BT, allowing for the development of
local proinflammatory immune response. The elimination of the pathogen results in the restoration of SCFAs-producing species and the previous host-microbiota equilibrium (181).

**MICROBIOTA DYSBIOSES AND INFLAMMATORY BOWEL DISEASE**

Hippocrates said “all disease begins in the gut” and over 2000 years later we are beginning to appreciate his sentiment. Gut microbiota dysbiosis, i.e. a microbial community imbalance (qualitatively and/or quantitatively), have been suggested to play a role in the development of some proinflammatory diseases, such as IBD (245-247), type 1 diabetes, allergy, asthma, rheumatoid arthritis, and neurological diseases (248, 249). IBD refers to a heterogeneous group of disorders with high rate of incidence in the world and associated with chronic inflammation of the gut (250). Although the cause of IBD remains unknown, considerable progress has been made in the recent years to understand the disease pathogenesis. IBD appears to be triggered by a combination of genetic, environmental and immunologic factors in which an uncontrolled immune response lead to development of chronic inflammation (251). In general, IBD is characterized by an infiltration of large numbers of inflammatory cells in intestine, leukocytosis, abdominal pain, diarrhea and weight loss. However, have the tendency to shift from periods of active disease to periods of little or no disease activity (remission). A large number of environmental factors, such as microbiota composition, infections, diet, drugs, stress, and socioeconomic status have been implicated in the pathogenesis of IBD (251). In addition a large number of genetic susceptibility genes to develop IBD are also involved in immune response to microbiota (e.g. NOD2, TLR4, CARD9, ATG16L1, IL23R polymorphisms) (251, 252). The most common forms of IBD are Crohn’s disease (CD) primarily affects the small intestine and colon and ulcerative colitis (UC) that is limited to the colon (253). Although CD and UC have been studied together because they share common features (such as symptoms and structural damage), it is now clear that they represent
two distinct diseases. For example, the genetic associations are different in both conditions (252) and microbiota has an important role in CD pathogenesis (245, 246), but less relevance in UC (247, 252). CD was designated as a Th1 condition, whereas UC is associated with an exaggerated Th2 mediated response (253). However, this classic view of Th1/Th2 has been challenged because CD and UC have also increased number of Th17 cells and an interplay among Th1, Th2, Th17 and Tregs cells (254). IBD has linked to marked increase in the number of effector immune cells such as proinflammatory TCRαβ⁺ IELs (255), macrophages (178), dendritic cells (256, 257), neutrophils (187), mast cells (258), ILCs (259-263), activated lamina propria αβ T (264, 265) and γδ T cells (266-268). The increase of effector cells IBD patients may be explained partially by an enhanced influx of these cells into the intestinal wall in response to an increase in cellular adhesion ligands and chemoattractants within the inflamed mucosa (269, 270). Cytokines play a crucial role in wound healing and intestinal inflammation resolution. Therefore, the increase of pro-inflammatory and decrease of anti-inflammatory cytokines that occurs in IBD results in tissue damage, disease progression and limits the resolution of inflammation (254).

IBD patients have frequently infections with some opportunistic pathogens suggesting that these bacteria can initiate disease (Table 1) (271). Although these alterations in microbiota composition have long been associated with human IBD pathogenesis, it should be emphasized that the studies discussed so far describe associations and do not prove causation. However, in animal models the acute infection with some pathogens induce microbiota dysbiosis, a dramatic impact on the structure and function of gut immune system, promoting chronic gut inflammation (271-273). An alternative hypothesis suggests that the reduction in the number of tolerogenic bacteria of the phyla Bacteroidetes and Firmicutes (1, 274-278), particularly the Firmicutes, Clostridium clusters IV and XIVa, e.g. Faecalibacterium prausnitzii (279-282), can induce IBD (Figure 2). For example during anaerobic growth Faecalibacterium prausnitzii
species produce substantial quantities of BT, formate, small amounts of D-lactate and other metabolites that would play a major role in the suppression of inflammation (245). The studies that describe associations of microbiota dysbiosis with IBD pathogenesis do not provide information about the timing of dysbiosis relative to disease onset and should be interpreted with caution particularly with regards to cause-effect relationships. However, IBD patients have reduced numbers of SCFAs-producing bacteria (Bacteroidetes and Firmicutes) (94) and SCFAs concentration in feces (275, 283-285). In line with this, the administration of probiotic bacteria with the capacity to produce SCFAs has been shown to decrease proinflammatory microenvironment in animal models of IBD (286, 287) and the administration of SCFAs has been used with positive results in the reduction of human colitis severity (288-291). To exert their anti-inflammatory effect in immune cells, SCFAs needs to be absorbed in gut epithelium by two specific transporters, monocarboxylate transporter 1 (MCT1) and/or sodium-coupled monocarboxylate transporter 1 (SMCT1) (139). However, these transporters are downregulated during inflammation and in inflamed mucosa of IBD patients (292-294). The treatment of IECs with proinflammatory mediators (IFN-γ and/or TNF-α) decreased the expression of MCT1 and SMCT1 (292-294), suggesting that the intense inflammatory milieu during IBD causes a decrease in the expression of BT transporters. Therefore, IBD patients showed microbiota dysbiosis, reduced concentration of BT in gut lumen (275, 283, 284) and decreased absorption in gut epithelium (292-294). As described above BT have anti-inflammatory effect in various components of the immune system implicated in gut inflammation: the innate (i.e. intestinal epithelial cells, macrophages, dendritic cells and neutrophils) and adaptive (T and B cells), suggesting that microbiota dysbiosis and reduced gut BT concentration may be involved in IBD pathogenesis. For example, IBD patients showed an increased number of Tregs in inflammatory lesions compared to healthy controls (295-297). Tregs are not able to control the inflammation despite being present in increased numbers. tTregs constitutively expressed Foxp3 (74) and
under normal circumstances tTregs cells are very stable and long-lived (298). In contrast, Foxp3 expression is less stable in pTregs (59, 299-301). Acetylation of Foxp3 appears to be required for optimal Foxp3 stability and enhanced activity of Tregs (302). BT increases Foxp3 acetylation and can enhance pTregs stability and activity (94, 209). Therefore, microbiota dysbiosis and decrease in BT concentration can induce loss of pTregs numbers and/or suppressive activity (74). pTregs have also plasticity to differentiate to other Th types under inflammatory stimuli (59, 299-301). Moreover, is also important to understand if the decrease in BT concentration observed in IBD patients have a role in the acquisition of Tregs proinflammatory capacity. Besides, SCFAs can regulate host systemic immune system (88) and reduced numbers of SCFAs-producing bacteria and SCFAs concentration in gut (275, 283-285) can affect also the development of other systemic proinflammatory diseases such as type 1 diabetes (303), lung allergic inflammation (15, 98, 99) and neuroinflammation (89, 100, 101).

CONCLUSIONS AND FUTURE PROSPECTS FOR THERAPY

The relationship between microbiota dysbiosis and IBD is complex and dynamic, rather than one of simple cause-effect. Studies attempting to determine whether microbiota dysbiosis is truly causative or merely a consequence of inflammation in humans, have suffered from a number of limitations, making it difficult to have definitive conclusions. However, IBD patients have microbiota dysbiosis with reduced numbers of SCFAs-producing bacteria and reduced concentration of BT in the gut. BT mediates increase in anti-inflammatory cells (e.g. Tregs, Tr1 and Bregs) and decreases proinflammatory mediators (e.g. macrophages, dendritic cells, neutrophils and effector T cells) in gut, suggesting that microbiota dysbiosis and reduced gut BT concentration are involved in the pathogenesis of IBD. Already in early 1900, commensal microbes have been demonstrated to have beneficial properties in the host, and Ilya Mechnikov had proposed the use of live microbes to maintain gut health and prolong life. Therefore,
probiotic supplementation (e.g. orally administered capsules with BT producing bacteria) and modulation of diet (increased prebiotics - fiber) can be one option to treat IBD patients. Another option for manipulation of the gut microbiota involves fecal microbiota transplantation (FMT) from healthy donors to IBD patients (304). Interestingly enough, the richness of donor microbiota with BT-producing bacteria is correlated with successes of FMT (305). Given its anti-inflammatory effect, BT have been proposed to be used in IBD therapy (306). However, the patenting of a natural product has an adverse impact on its clinical development. Some structural analogs of BT were developed, e.g. Tributyrin (307) and AN-9 (308), and some strategies are currently being developed to improve bioavailability of these compounds to use in clinical trials (307, 309).

To end with Louis Pasteur: “Gentlemen, it is the microbes who will have the last word”.

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CONFLICT OF INTEREST
No conflicts of interest are declared by the author(s).

LIST OF ABBREVIATIONS
BT - butyrate
CD - Crohn’s disease
FMT - fecal microbiota transplantation
GALT - gut-associated lymphoid tissue
GF - germ-free
GPR - G-protein-coupled receptor
HDACi - histone deacetylases inhibitor
| 1  | IBD - inflammatory bowel disease |
| 2  | IEC - intestinal epithelial cell  |
| 3  | Ig – immunoglobulin              |
| 4  | ILC3s - group 3 innate lymphoid cells |
| 5  | LNs - lymph nodes                |
| 6  | LTi - lymphoid tissue inducer    |
| 7  | MAIT - mucosal-associated invariant T cells |
| 8  | MAMP - microbe-associated molecular pattern |
| 9  | MCTs - monocarboxylate transporters |
| 10 | NF-κB - nuclear factor-kappa B   |
| 11 | NODs - nucleotide-binding oligomerization domain |
| 12 | Nrp1 - neuropilin-1              |
| 13 | SCFAs - short-chain fatty acids  |
| 14 | SMCT1 - sodium-coupled monocarboxylate transporter 1 |
| 15 | Th - T-helper                    |
| 16 | TLR - toll-like membrane receptors |
| 17 | Tr1 - CD4⁺Foxp3⁺IL10⁺            |
| 18 | Tregs - regulatory T cells       |
| 19 | UC - ulcerative Colitis         |
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Figure 1 - The crosstalk between microbiota and the immune system: butyrate (BT) take center stage. BT function directly on intestinal epithelial cells, where they serve as an energy source and have an anti-inflammatory effect. In addition, act directly on immune cells, BT inhibit T cells recruitment, proliferation and induced apoptosis, inhibits T cell activation and increases the number and activity of immunosuppressive Tregs. BT inhibits activation and activity of neutrophils, macrophages, dendritic cells and T cells, thereby eliminating the source of inflammation in the gut tissue. These findings elucidate pathways in which the BT mediates down-regulation of proinflammatory effectors and suggest that microbiota dysbiosis and reduced gut BT concentration may be involved in the pathogenesis of IBD.

Figure 2 - Microbiota in intestinal homeostasis and inflammation. In healthy conditions the higher diverse commensal gut microbiota community is able to ferment dietary fiber releasing short-chain fatty acids (SCFAs). SCFAs, particularly butyrate (BT), regulates innate and adaptive immune cell generation, trafficking, function, and has an anti-inflammatory effect. This homeostasis is disrupted in inflammatory bowel disease (IBD) patients that have frequently infections with some opportunistic pathogens and microbiota dysbiosis, i.e. reduced numbers of SCFAs-producing bacteria and reduced concentration of BT in the gut. This has been linked to a marked increase in the number of proinflammatory immune cells in the gut mucosa of these patients, suggesting that reduced gut BT concentration may be involved in IBD pathogenesis.
Figure 1
Figure 2

- diverse commensal microbiota
- 90% *Firmicutes* and *Bacteroidetes*
- enhanced SCFAs production
- no over inflammation
- intact mucosal barrier

healthy state

- reduced microbiota diversity
- increase in opportunistic pathogens
- reduced *Firmicutes* and *Bacteroidetes*
- reduced SCFAs production
- intestinal inflammation
- disrupted mucosal barrier

Generation of Dysbiosis
- host genetic factors
- environmental factors
- infections

Dysbiosis