



HAL
open science

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

Pedro Gonçalves, João Ricardo Araujo, James P Di Santo

► **To cite this version:**

Pedro Gonçalves, João Ricardo Araujo, James 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. *Inflammatory Bowel Diseases*, 2018, 24 (3), pp.558-572. 10.1093/ibd/izx029 . pasteur-02122775

HAL Id: pasteur-02122775

<https://pasteur.hal.science/pasteur-02122775>

Submitted on 7 May 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3

4 **Pedro Gonçalves^{1,2*}, PhD; João Ricardo Araújo^{3,4}, PhD; James P Di Santo^{1,2}, MD, PhD.**

5 ¹Innate Immunity Unit, Institut Pasteur, Paris, France and ²Institut National de la Santé et de la
6 Recherche Médicale (INSERM) U1223, Paris, France; ³Molecular Microbial Pathogenesis Unit, Institut
7 Pasteur and ⁴Institut National de la Santé et de la Recherche Médicale (INSERM) U1202, Paris, France;

8 ***Corresponding author:** Pedro Gonçalves; Innate Immunity Unit, Institut Pasteur, Paris, France and
9 Institut National de la Santé et de la Recherche Médicale (INSERM) U1223, Paris, France; Tel.:
10 00330601080704, Email: pedro.goncalves@pasteur.fr

11 **Source of funding:** Pedro Gonçalves is currently receiving a grant from the “Labex Milieu Intérieur”
12 ANR 10-LBX-69 MI (<http://www.milieuinterieur.fr/en>). Research conducted in the Di Santo
13 laboratory is supported, in part, by grants from the Institut Pasteur, INSERM, ANR
14 (ILC3_MEMORY) and ERC (ILC_REACTIVITY).

15

1 **ABSTRACT**

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

18

19 **Keywords:** microbiota, immune system, short chain fatty acids, butyrate, inflammation,
20 inflammatory bowel disease.

1 **HOST COLONIZATION BY MICROBIOTA**

2 The body of mammals is normally colonized by bacteria, fungi, and viruses, collectively
3 referred as the commensal microbiota. In humans, although distinct microbiota inhabit all body
4 surfaces exposed to the environment, the largest (10^{14} microbes) and most varied microbiota
5 population (100-1000 species) resides in the gut (1). Microbial density increases along the gut,
6 with few bacteria in the upper small gastrointestinal tract until it reaches its peak in the colon,
7 which contains the highest microbial diversity and load (up to 10^{12} bacteria/gram of faeces) (2).
8 Despite considerable individual variation (3), $\approx 90\%$ of adult gut microbiota belong to major
9 bacterial phyla gram-negative *Bacteroidetes* (*Bacteroides*, *Prevotella*, and *Porphyromonas*)
10 and gram-positive *Firmicutes* (*Clostridia* and *Bacilli*) (4). The highly stable and shared
11 component of microbiota between individuals is known as “core microbiota”, as opposed to a
12 highly variable component that is capable of fluctuate very fast in response to external stimuli
13 and to adapt to new conditions (5).

14 Microbiota form a symbiotic relationship with their host: microbes benefit from the
15 nutrient-rich environment of the gut, and on the other hand, microbiota encode hundreds of
16 genes that are absent in the human genome and modulate several biological functions of the
17 host, including nutrient processing, maintenance of energy homeostasis and immune system
18 development (6). Despite bacterial transmission from the mother to the fetus can occur during
19 human pregnancies (7), the greatest microbiota colonization is initiated by maternally derived
20 microbes at birth (8). The delivery mode is a major determinant of the newborns microbiota
21 composition, babies delivered by cesarean section (C-section) acquire a microbiota that is
22 enriched in mother’s skin microbes while vaginally delivered babies acquire a microbiota
23 resembling those of the maternal vagina (9). The microbiota colonization during the first weeks
24 of newborn life plays a essential role in the development and education of the host mammalian
25 immune system (10) and babies delivered by C-section has been associated with increased risk

1 for development of obesity, asthma, allergies and immune deficiencies (11). However, the
2 composition of microbiota community is rapidly evolving during the first year of life, when
3 *Bifidobacterium* is dominant, microbial diversity gradually increases, reaching an adult stable
4 configuration at age of 2-3 years (4). During this evolution the host genetics can shape the
5 microbiome composition (12), but the environmental factors (e.g. dietary and antibiotic use)
6 have a major impact in shaping this community (4, 8). The adult microbiota composition of C-
7 section delivered babies is comparable to vaginally delivered (3, 13), suggesting that delivery
8 mode does not affect adult microbiota composition. Dietary and microbial antigens can also be
9 absorbed in the mucosa, transferred across the placenta from the mother blood to the fetus and
10 affect the development of fetal immune system. For example, maternal consumption of vitamin
11 A and subsequent generation of retinoic acid signalling is important for modulation of fetal
12 lymphoid tissue inducer (LTi) cell differentiation which impacts on the size of secondary
13 lymphoid organs and the efficiency of immune responses in the adult offspring (14).
14 Metabolites produced by microbiota during late pregnancy protect also against the development
15 of allergic responses in adult offspring (15). Recently, a study in pregnant mice colonized with
16 the bacteria that then returned to a germ-free (GF) state before giving birth, showed that
17 microbiota metabolites produced in pregnancy leads to increased numbers of group 3 innate
18 lymphoid cells (ILC3s) and intestinal mononuclear cells in gut of newborns (16). This increase
19 persisted until at least 8 weeks after birth, suggesting that temporary microbiota colonization
20 has long-term effects on the immune system. Maternally acquired antibodies during pregnancy
21 specific from commensal microbes can also regulate T helper (Th) cell activation in gut of
22 neonatal mice (17). Therefore, the exposure to various microbial antigens, metabolites and
23 antibodies in the fetus contributes to the early development of a functional immune system (7).
24 These studies suggests that both prenatal (maternal) and the early life (infant) microbiota have
25 crucial roles in the development of immune system.

1 Decades of studies in GF animals showed that multiple microbial species have an
2 important role in the development of gut-associated lymphoid tissue (18-20) and in the
3 colonization of the gut wall by immune cells, such as the so-called intraepithelial lymphocytes
4 (21-24), lamina propria neutrophils (25), dendritic cells (26), ILC3s (16, 27, 28), mucosal-
5 associated invariant T (MAIT) cells (29, 30), TCR $\alpha\beta$ Th17 cells and TCR $\gamma\delta$ IL-17-producing
6 cells (31-33), regulatory T cells (Tregs) (34), and immunoglobulin (Ig)A secreting plasma cells
7 (35, 36). Moreover, the maturation of specific immune cells depends on certain host-specific
8 bacterial species (26). For example, particular bacteria have been linked to the development of
9 Th17 cells (37, 38) and others are involved in the expansion of Foxp⁺ Tregs (34, 39). Despite
10 these studies in mice there are no studies about the effect of microbiota in human cellular subset
11 composition (40). However, human healthy individuals have high level of interindividual
12 variation in immune cell subset composition and the largest influence on immunological
13 variation identified was cohabitation, with 50% less immune cell variation between individuals
14 who share the same environment (41). Microbiota composition may have a role, for example
15 cohabiting individuals show convergence in microbiomes (42, 43) possibly via direct
16 transmission (44). Microbiota protect also the host from opportunistic pathogenic infection (45,
17 46), since they inhibit the adhesion to the gut mucus and colonization by pathogens (47). In
18 addition to this barrier effect, commensal microbiota produce bacteriocins and short chain fatty
19 acids (SCFAs), compounds that are able to inhibit the growth of potentially invading pathogenic
20 bacteria (47, 48). Louis Pasteur two centuries ago already said that “the role of the infinitely
21 small in nature is infinitely great”.

22

23 **MICROBIOTA AND THE GUT IMMUNE SYSTEM**

24 Gut-associated lymphoid tissue (GALT), together with intestinal draining lymph nodes
25 (LNs), serve as the major sites of adaptive immune cell priming. Antigens sampled through M

1 cells are taken up by antigen-presenting cells for processing and presentation to lymphocytes
2 in gut-associated lymphoid tissue. In addition, some bacteria can directly colonize gut-
3 associated lymphoid tissue (i.e. lymphoid follicles, Peyer's patches and the mesenteric lymph
4 nodes) in healthy mammals. (49-51).

5 The majority of adaptive immune cells in the epithelium are intraepithelial lymphocytes
6 (IELs) (52). These cells are located between intestinal epithelial cells (IECs) in a ratio of
7 approximately one lymphocyte for every six to ten IECs (53). For example, small intestine
8 harbor ≈ 50 millions intraepithelial T lymphocytes (IELs) representing up to half the number
9 of T cells in the mice (54). IELs include conventional $CD8^+$ and $CD4^+$ $\alpha\beta$ T cells, as well
10 unconventional T cell types including $TCR\gamma\delta^+$ and $CD8\alpha\alpha^+$ $TCR\alpha\beta^+$ lymphocytes (53). $TCR\gamma\delta^+$
11 constitute a major proportion of the IELs population that contributes to the maintenance of the
12 epithelial barrier and gut homeostasis (53). Conventional $TCR\alpha\beta^+$ IELs derive from naïve T
13 cells that have undergone activation in GALT or LNs and subsequently homed to the intestine
14 to generate epithelial resident effector memory T cell population. Microbiota diversity induces
15 a progressive and considerable increase in the numbers of both $CD8\alpha\beta^+$ and $CD8\alpha\alpha^+$ $TCR\alpha\beta^+$
16 IELs (26, 55, 56). The absence/reduction of $CD8\alpha\beta^+$ $TCR\alpha\beta^+$ IELs in GF mice (21-24) supports
17 a major role of the microbiota in the generation of these cells (22, 57, 58). Underlying the
18 intestinal epithelium is the lamina propria. The most abundant immune population in the lamina
19 propria is macrophages, which rapidly phagocytize microbes that breach the gut physical
20 barrier. These macrophages predominantly represent tissue-resident non-migratory
21 macrophages, belonging to the functional M2 subtype characterized by the production of large
22 amounts of IL-10. Besides macrophages, lamina propria is also rich in dendritic cells. These
23 cells can extend dendrites between IECs without disrupting the intestinal barrier function,
24 acquire antigens from microbiota and subsequently migrate to mesenteric lymph nodes, where
25 they are presented these antigens to T cells. In normal conditions, dendritic cells induce

1 tolerance to commensal microbiota antigens, through the secretion of TGF- β and retinoic acid
2 induces de differentiation of naïve CD4⁺ T cells in Tregs (59).

3 Part of the adaptive immune system, B cells are one of the predominant population in
4 the lamina propria of small intestine and colon (60). Was estimated that 80% of human and
5 mouse fully differentiated B cells (plasma cells) are located in the intestinal lamina propria.
6 One of the most prominent effects of microbiota colonization is the induction of secretory IgA
7 (35, 36). The switch of IgM-positive B cells to IgA-positive B cells occur mostly in GALT, a
8 T dependent and independent pathway, induce the production of IgA that is next secreted at the
9 epithelial level. IgA binds to surface molecules expressed by microbes causing their
10 agglutination in the gut lumen (61). High-affinity antigen-specific IgA, produced by a T
11 dependent pathway, binds to surface molecules expressed by pathogenic bacteria and their
12 toxins and helps in their neutralization (62-64). On the other hand, commensal bacteria might
13 also become coated with IgA but the level of these process is generally been believed to be
14 lower as compared to pathogenic bacteria (65). This is probably due to the fact that commensal
15 microbiota induces low-affinity IgA in a T cell independent manner, since mice lacking cognate
16 T-cell-B-cell interactions or mice lacking T cells still mount IgA responses to commensal (62-
17 64).

18 Lamina propria is also rich in conventional $\alpha\beta$ T cells, \approx 15% of CD8⁺ and \approx 85% of
19 CD4⁺ T cells in mice (38). CD4⁺ T cells in the lamina propria of small intestine and colon are
20 predominantly ROR γ t⁺ Th17 cells and ROR γ t⁺ Foxp3⁺ Tregs (66). ROR γ t⁺ Th17 represents
21 40% of CD4⁺ T cells in the small intestinal (38) and about 50% in the colonic lamina propria
22 (67). Tregs represents 20% of CD4⁺ T cells in the small intestinal lamina propria and about
23 30% in the colonic lamina propria (68). Microbiota colonization is a prerequisite to gut
24 accumulation of both ROR γ t⁺ Th17 cells (31, 67, 69) and Foxp3⁺ Tregs (34, 70, 71). Microbiota
25 induces antigen-specific ROR γ t⁺ Th17 cells responses in the gut (72), and these cells are also

1 essential for protection against opportunistic infections. On the other hand, Tregs suppress a
2 wide range of immune cells, maintain tolerance to environmental antigens and avoid the risk of
3 gut inflammation (73). These cells are characterized by the permanent expression of high levels
4 of the transcription factor Foxp3, which is considered to confer their suppressive phenotype
5 (74). The majority of Foxp3⁺ Tregs develop in the thymus - thymus-derived Tregs (tTreg) -,
6 alternatively naïve CD4⁺ T cells upon antigen exposure and in the presence of TGF-β and
7 retinoic acid can also be induced to express Foxp3, producing peripherally-induced Tregs
8 (pTregs) in extrathymic tissues (75). Some studies suggest that a substantial proportion of the
9 colonic Tregs arises extrathymically from antigen-specific interactions with the colonic
10 microbiota (34, 70, 71). These pTregs express a unique subset of TCRs (which do not overlap
11 with the tTreg TCR repertoire) and recognized epitopes derived from commensal microbiota
12 (70, 71).

13 Innate lymphoid cells (ILCs) are resident innate cells in the lamina propria that share
14 functional characteristics with differentiated T cells. ILCs can be subdivided into three different
15 groups referred to as group 1, 2, and 3. Group 3 ILCs in mice is further categorized in CCR6⁺
16 LTi cells and CCR6⁻ ILC3s cells, some CCR6⁻ ILC3 cells express a natural cytotoxicity-
17 triggering receptor (NCR, NKp46) and others not. At the present time, ILC3s appears to be the
18 most dependent on the presence of microbes. Microbiota induces CCR6⁺ LTi cells that promote
19 the formation of secondary lymphoid organs (18-20) and NCR⁺ ILC3s numbers (16, 27, 28).
20 Both CCR6⁺ ILC3 cells and NCR⁺ ILC3 cells are major producers of IL-22 and IL-17A,
21 cytokines that sustain epithelial integrity and regulate mucosal immunity (76). Microbiota
22 colonization stimulates IL-22 production by ILC3s (16, 27, 28). IL-22 maintains the intestinal
23 barrier function by promoting the production of antimicrobial peptides and mucus involved in
24 antimicrobial host defense, and promote wound healing, tissue remodeling and repair (28, 77,
25 78). IL-22 induces also fucosylated carbohydrate synthesis in IECs (79) and these

1 carbohydrates can supplies a selective advantage to beneficial commensal bacteria that can
2 metabolize these compounds (79, 80). On the other hand, IL-17 is essential for regulating
3 mucosal host defense against invading pathogens, and the disruption of this signaling in the gut
4 epithelium results in microbiota dysbiosis (81). ILC3s have other important functions, namely
5 MHCII⁺ ILC3s reduce commensal-specific Th responses by the antigens presentation and
6 induction of a CD4⁺ T cells negative selection process (82). In addition, ILC3-mediated release
7 of granulocyte/macrophage colony-stimulating factor via IL-1 β maintains colonic Tregs
8 homeostasis (83). Furthermore, ILC3-derived lymphotoxins orchestrate IgA secretion and
9 control microbial composition in the gut (84). All of these properties makes that ILC3s
10 depletion promotes the dissemination of bacteria in gut and drive systemic inflammation (28,
11 49, 85).

12

13 **MICROBIOTA AND SHORT CHAIN FATTY ACIDS**

14 The innate immune cells and non-immune cells of the host receive direct stimulus from
15 the microbial antigens through microbe-associated molecular patterns (MAMPs), such as toll-
16 like membrane receptors (TLRs) and intracellular nucleotide-binding oligomerization domain
17 (NODs). The continuous intestinal absorption of low-level microbial products such as NODs
18 and TLRs ligands, microbial antigens into the systemic circulation represents a crucial aspect
19 of the cross-talk between the microbiota and the systemic immune system (45). For example,
20 bone marrow progenitor cells can directly sense microbiota signals (e.g. LPS) via TLRs (86)
21 and microbiota can control immunity through regulation of hematopoiesis at primary immune
22 sites (87). Indeed, GF mice display reduced proportions and differentiation potential of specific
23 myeloid progenitors cells, have decrease resident myeloid cell populations in bone marrow and
24 reduction of in monocytes, macrophages and neutrophils in peripheral sites (87). On the other
25 hand, microbiota colonization can enhance myelopoiesis and increase the myeloid cells in

1 peripheral sites (87). Microbiota can also affect TCR $\alpha\beta^+$ CD8 $^+$ and CD4 $^+$ T cells, TCR $\gamma\delta^+$ and
2 ILCs in other peripheral sites (e.g. lymph nodes, lung, skin and brain) (88, 89) and regulate
3 immune responses in peripheral sites (e.g. in pancreas (90) and brain (89)).

4 Commensal microbial metabolism produces essential vitamins (e.g. group B and K
5 vitamins), SCFAs as well metabolizes compounds (e.g. bile acids, sterols and xenobiotics) that
6 are transformed into secondary bioactive metabolites and contribute to the gut “metabolome”
7 (4, 91). Besides structural components of bacteria, many of metabolites derived from
8 commensal microbial metabolism have been implicated in the development and function of
9 immune cells, e.g. vitamin B-based metabolites in MAIT cells (30, 92) and sphingolipids in
10 invariant Natural Killer T (iNKT) cells (93). However, the best-studied microbial metabolites
11 that influences immune system homeostasis are SCFAs, in particular acetate, propionate and
12 butyrate (BT) (Figure 1). Moreover, the most abundant commensal bacteria in gut,
13 *Bacteroidetes* and *Firmicutes*, are able to ferment non-digestible carbohydrates releasing
14 SCFAs (94) that can be found at high concentrations in healthy gut (e.g. 20 mM BT in colonic
15 lumen) (95). Among SCFAs, BT has multiple regulatory roles at gut level, exerting an anti-
16 inflammatory effect on both intestinal epithelial cells and immune cells (96, 97). This article
17 highlights our current understanding of how SCFAs, specifically BT, interact with immune cell
18 generation, trafficking, and function, and how these interactions influence host gut health
19 (Figure 1). SCFAs produced by the microbiota can also translocate from the gut to the
20 bloodstream and regulate host systemic immune system (88). For example, SCFAs can inhibit
21 hematopoiesis and inducing accumulation of immature dendritic cells in bone marrow (87, 98),
22 can increase Tregs in lungs (15) and protect against the development of lung allergic
23 inflammation (15, 98, 99). SCFAs are also key molecules that modulate microglia (the brain’s
24 macrophages) maturation, morphology and function (100) and can attenuate
25 neuroinflammation (101).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

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 transcytosis 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

1 chemokine and cytokine signaling. IECs can produce some cytokines (e.g. IL-10 and TGF- β)
2 that affect immune cells (118-120) and respond to cytokines and other mediators produced by
3 immune cells in the lamina propria (117) (Figure 1). IECs receive direct stimulus from the
4 microbial antigens through (TLR)1-5 and 9 and NOD2 (121). The activation of these receptors
5 induces the production of proinflammatory cytokines (122) and chemotactic factors for both
6 myeloid and lymphoid cells (123). However, IECs express low levels of TLRs on the apical
7 side (pole facing the intestinal lumen) and higher levels on the basolateral side (that
8 communicates with lamina propria), being essentially stimulated by invasive bacteria (121).
9 Interestingly, BT decreases TLR4 expression and production of proinflammatory cytokines in
10 IECs (124, 125). On the other hand, proinflammatory cytokines can drive the expression of
11 neutrophil chemoattractant chemokines in IECs (126), such as IL-8, a chemokine that BT can
12 significant decrease the secretion (124, 127). BT was also shown to inhibit the expression of
13 the chemokines CXCL5 and CXCL11 (128). In conclusion, BT has a direct anti-inflammatory
14 effect on IECs and in the crosstalk between IECs and gut immune cells.

15

16 **SCFA CROSSTALK WITH THE IMMUNE SYSTEM**

17 Understanding the complex molecular mechanisms underlying the anti-inflammatory
18 effect of SCFAs, specifically BT, has been difficult because they interact with multiple
19 signaling molecules. First, BT was demonstrated to be a physiologic ligand of membrane G-
20 protein-coupled receptors GPR109A (129), GPR41 and GPR43 (130-132). GPR109A are not
21 activated by propionate and acetate. However, acetate can activate GPR43 and propionate both
22 GPR41 and GPR43, although with different affinities (129-132). GPR109A is a receptor
23 expressed by some immune cells (e.g. macrophages, dendritic cells and neutrophils) and the
24 activation of this receptor decrease the production proinflammatory mediators (133, 134).
25 GPR41 and GPR43 are also expressed by some immune cells such as monocytes, dendritic

1 cells, eosinophils and neutrophils (130, 131, 135-138). At least GPR43 seems to be important
2 in the regulation of immune responses, since mice lacking GPR43 develop exacerbated
3 inflammation in models for colitis due to higher production of inflammatory mediators and
4 increased recruitment of immune cells (135, 136). SCFAs uptake inside the immune cells is
5 dependent on monocarboxylate transporters (MCTs) (139). Secondly, intracellular BT (and
6 propionate but not acetate) control gene expression by direct inhibition of histone deacetylases
7 (HDACs) (140-144). BT inhibits the activity of most zinc-dependent HDACs, except class IIa
8 HDACs 6 and 10 isoforms, (140-144) inducing the hyperacetylation of histones, resulting in
9 open structure of chromatin and leading DNA accessible to initiate transcription of genes. BT
10 acting as a histone deacetylases inhibitor (HDACi) can overcome the inflammatory response
11 by inhibition of nuclear factor kappaB (NF- κ B) activation and production proinflammatory
12 mediators (97, 145). Synthetic HDACi are being developed for treatment of proinflammatory
13 diseases such as inflammatory bowel disease (IBD) (146). BT can also induce the expression
14 of nuclear receptor peroxisome proliferator activated receptor (PPAR) γ (147), which exerts
15 anti-inflammatory effects through antagonism of NF- κ B (148). However, BT can induce other
16 epigenetic modifications, namely hyperacetylation of nonhistone proteins (149), selective
17 inhibition of histone phosphorylation (150), histone methylation (151) and DNA methylation
18 (152). Third, metabolism has a direct role in regulating immune cell function. BT can be
19 metabolized in tricarboxylic acid cycle, inhibiting glycolysis (153) and inducing autophagy
20 (154). BT increase Akt/mTOR activity a pathway implicated in the regulation of immune
21 responses (155). Therefore, the integration of BT into cellular metabolism can regulate cell
22 energy status and several signaling processes (156, 157).

23

24 ***Immune cell differentiation and recruitment***

1 Lymphoid and myeloid cells develop largely from hematopoietic stem cells resident in
2 bone marrow. As previously discussed, SCFAs can inhibit hematopoiesis (87, 98), particularly
3 myelopoiesis and decrease resident mature myeloid cell populations in bone marrow (15, 98).
4 Consistent with these data, *in vitro*, BT decreases the differentiation of monocyte to derived
5 macrophages (158) and dendritic cells (159-161). BT impairs the differentiation and maturation
6 of dendritic cells by its HDACi activity (162, 163). On the other hand, histone modifications
7 are essential for B cell development and proliferation (164), BT acting at this level can modulate
8 B cell differentiation and induce B cell accumulation (165, 166). In addition, BT can affect the
9 recruitment of immune cells to the periphery. Monocyte chemoattractant protein-1 (MCP-1) is
10 one of the key chemokines that regulate migration and infiltration of macrophages into the gut,
11 BT reduces the release of MCP1 and vascular cell adhesion molecule-1 (VCAM1) (167, 168),
12 decreasing the recruitment and migration of macrophages (168, 169). Macrophages and
13 dendritic cells can induce the recruitment of leukocyte (chemotaxis) (170). BT reduce the
14 release of several proinflammatory chemokines from human monocyte-derived dendritic cells
15 (e.g. CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11) (171) and IECs (e.g. CXCL5 and
16 CXCL11) (128) and can modulate leukocyte trafficking. BT reduces surface expression of
17 chemoattractant receptors C5aR and CXCR2 in neutrophils and inhibits their chemotaxis *in*
18 *vivo* acting through GPR43 receptor (136). Therefore, BT decreases neutrophils recruitment *in*
19 *vivo* to the gut (125, 172). BT treatment reduces also the number of neutrophils in crypt and
20 surface epithelia of IBD patients (145). Interestingly enough, BT reduces the recruitment of
21 circulating T cells to the inflammatory sites (136, 173). The surface expression of
22 chemoattractant receptors CXCR2 in lymphocytes is reduced by BT treatment, contributing to
23 a decrease in T cells migration to inflammatory sites (136). On the other hand, compounds with
24 HDACi activity stimulates thymic production of tTregs and increases their peripheral numbers
25 in normal mice (174). BT can also increase tTregs numbers in colon (138). The oral

1 administration of SCFAs can mediate the migration of tTregs into the gut through the
2 upregulation of GPR15 receptor, a receptor implicated in the homing of mice tTregs in colon
3 (138, 175, 176). GPR43 and GPR41 receptors are also expressed in leukocytes (130, 137).
4 SCFAs induced lung Tregs suppressor function by a GPR41-dependent manner (177),
5 suggesting that these receptors may have a chemoattractant effect for Tregs.

6

7 ***Decrease in proinflammatory mediators***

8 Macrophages through the secretion of cytokines can control the inflammatory state of
9 the gut (178). The activation and production of proinflammatory cytokines by macrophages is
10 regulated by histone modifications (179, 180). Therefore, BT acting as a HDACi, inhibits
11 macrophages-mediated production of proinflammatory mediators (e.g. TNF- α , IL-6) *in vitro*
12 (181, 182) and *in vivo* (94, 181). In addition, BT induces their apoptosis (183) decreasing the
13 number of proinflammatory M1 macrophages *in vivo* (145, 181). Dendritic cells are also able
14 to drive inflammatory T cell responses in gut. However, BT decreases dendritic cell co-
15 stimulatory molecules (184, 185), decreases their activation (107) and suppresses the
16 production of Th1 cytokines (e.g. TNF- α , IL-1 β , IL-8) (180, 184, 186). Neutrophils can also
17 release multiple proinflammatory mediators (187). BT reduces the production of
18 proinflammatory cytokines (e.g. TNF- α and cytokine-induced neutrophil chemoattractant-2) in
19 neutrophils (136, 173, 188), decreases their phagocytosis capacity and cytotoxic effect (189-
20 193). BT can induce apoptosis (194) and the induction of apoptosis might be involved the
21 observed decrease of neutrophil numbers *in vivo* (195). Mast cells are also traditionally
22 associated with inflammation. However, BT through HDACi activity has been shown to
23 inhibits cell proliferation, increases cell apoptosis and suppress mast cell proinflammatory
24 cytokines production (196).

1 The activation of naïve T cells requires presentation of antigens to T cells by antigen
2 presenting cells and, as described above, BT modifies the biology of antigen presenting cells.
3 Therefore, BT inhibits T cell activation (197) in response to antigens (197, 198) and mitogens
4 (199). Naïve T cells begin proliferating, before their acquisition of effector functions. However,
5 BT acting as a HDACi blocks cell cycle in G1 phase, inhibiting T cells cell-cycle progression
6 (184, 200-202) and the proliferation of both CD4⁺ and CD8⁺ T cells (96, 160, 203). On the
7 other hand, upon activation Th1, Th2 and Th17 cells use primarily glycolysis for energy
8 generation (204). Lactate anions produced during glycolysis are exported out of the effector T
9 cells by monocarboxylate transporters (MCTs) (205). MCTs are bidirectional transporters and
10 extracellular BT, by competing with intracellular lactate for MCTs, can lead to inhibition of
11 lactate efflux, induce intracellular accumulation of lactate, feedback inhibition of glycolysis
12 (153). Therefore, the integration of BT in T cell metabolism inhibits glycolysis (153) and Th
13 cell differentiation and proliferation (206). BT can also induce apoptosis of CD4⁺ and CD8⁺
14 effector T cells (207, 208). BT, in naïve CD4⁺ T cells, affects neither the acetylation nor the
15 expression levels of “masters” transcription factors T-bet, Gata3, and ROR γ t for the
16 development of Th1, Th2 and Th17, respectively (209). However, BT inhibited transducer and
17 activator of transcription (STAT)1 (210, 211) and STAT5 (212) signaling pathways in activated
18 CD4⁺ T cells and inhibits the production of Th1 (197, 198, 213, 214) and Th17 cytokines (163,
19 215). These data suggest that BT can inhibit the activation and production of proinflammatory
20 cytokines.

21

22 ***Increase in anti-inflammatory mediators***

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

1 generation of mouse and human Foxp3⁺ cells *in vitro*, increased pTregs subset *in vivo* in mice
2 colon lamina propria and reduces experimental colitis severity (94, 138, 209, 216-218).
3 Interestingly enough, other SCFAs are also able to induce Foxp3⁺ expression, promoting Tregs
4 accumulation in lungs and inhibiting the development of allergic responses (15). Recently, it
5 has also been shown *Clostridium* species produce SCFAs from butyrylated high-amylose diet
6 and induced specifically RORγt⁺ Tregs in mouse colon lamina propria (67). However, in
7 another study, SCFAs was quantified by liquid chromatography-mass spectrometry (LC-MS)
8 in cecal content and they did not found correlation between SCFAs levels in gut and RORγt⁺
9 Treg frequency (69), most probably because SCFAs are efficiently and quickly absorbed from
10 gut lumen to IECs (110). Butyrylated starches are chemically modified starches, in which the
11 acyl group is linked to the starch framework by an ester bond, found to be used by colonic
12 bacteria that deliver esterified BT and propionate that leads to changes in gut microbiota
13 composition. Therefore, normal dietary resistant and butyrylated starches can have different
14 effects on microbiota profiles and in the induction of RORγt⁺ Tregs. The induced RORγt⁺
15 pTregs have high levels of IL-10 production, a stable regulatory function even in inflammatory
16 conditions, (219) and can regulate Th1, Th2 and Th17 cell responses (69, 220). RORγt⁺ Tregs
17 are mostly negative for neuropilin-1 (Nrp1) and Helios expression and are not present in GF
18 conditions (67, 69, 219). Another question arising from these studies is whether the Foxp3⁺
19 Tregs induced by BT are antigen-specific. BT promotes the differentiation of colonic OVA-
20 specific OT-II Foxp3⁺ T cells following the adoptive transfer of naïve OT-II CD4⁺ T cells and
21 oral administration of OVA antigen (107, 209). On the other hand, the administration of BT
22 failed to increase colonic Tregs in GF mice, suggesting that for the induction of pTregs naïve
23 CD4⁺ cells need to recognize bacterial antigens (67, 69, 209). Therefore, Foxp3 expression
24 needs to synergize with TCR signals to the generation of functional pTregs (221) and BT only
25 “burst” the conversion and expansion of antigen-specific Foxp3⁺ pTregs.

1 BT facilitates pTregs differentiation by direct and indirect mechanisms. First, BT acting
2 directly in naïve CD4⁺ T cells as a HDACi enhance acetylation status of histone H3 in the
3 promoter and CNS3 enhancer regions of the *Foxp3* gene loci leading to expression of *Foxp3*
4 protein (94, 209). Compounds with HDACi activity increase *Foxp3* expression and enhance the
5 suppressive function of Tregs (222, 223). Second, TCR activation and induction of anergy in
6 peripheral CD4⁺ T cells, a state where the cell lose the ability to proliferate in response to
7 antigens, can gave rise to pTregs (224). In addition, inhibition of glycolysis promotes Tregs
8 differentiation (225). Therefore, BT induces anergy (197, 198, 213, 214) and blocks glycolysis
9 (153), promoting the generation of pTregs (225). BT can indirectly induce pTregs by acting in
10 dendritic cells and B cells (59). A study showed that the treatment of dendritic cells *in vitro*
11 with BT markedly enhanced the ability of dendritic cells to induce *Foxp3* expression in naïve
12 CD4⁺ T cells (94). Another study showed that BT induces *Raldh1* expression and promotes
13 retinoic acid production in dendritic cells (107). Lamina propria plasma cells can also produce
14 IL-10 (226), TGF-β and retinoic acid that induce *Foxp3*⁺ Tregs (227). BT switches the
15 differentiation of B cells into plasma cells to IL-10⁺ producing B cells (Breg) (228, 229).
16 Therefore, BT regulate the capacity of B cells to produce IL-10 and induce pTregs (230).

17 The anti-inflammatory cytokines, IL-10 and TGF-β, promote a microenvironment that
18 can modulate the function of other gut immune cells. For example, CD4⁺*Foxp3*⁺IL10⁺ (Tr1)
19 cells are present in high numbers in the intraepithelial and lamina propria of small intestine
20 (231). These cells are induced by chronic activation of CD4⁺ T cells by antigen in the presence
21 IL-10. However, BT treatment can induce directly by HDACi activity the development of Tr1
22 cells (107, 155). BT promotes also the increase in anti-inflammatory M2 macrophages (232-
23 234) acting directly in these cells as a HDACi (234) and indirectly by the induction of pTregs
24 (235) and production of Th2 cytokines, such as IL-10 (138, 188) and IL-5 (236). The anti-
25 inflammatory microenvironment can inhibit the expression of costimulatory molecules, MHCII

1 levels and proinflammatory cytokine production in dendritic cells, inducing tolerogenic
2 dendritic cells (237). Recently, it has been shown that SCFAs stimulate intestinal epithelial
3 cells to induce mucosal tolerogenic CD103⁺ dendritic cells (238). On the other hand,
4 tolerogenic dendritic cells can induce Foxp3⁺ Tregs, Tr1 cells and Bregs cells that produce the
5 immunosuppressive cytokines (237). ILC3s play also a key role in regulation of gut
6 homeostasis. BT promotes the production of retinoic acid in IECs (239) and dendritic cells
7 (107) that is important for modulation of ILC3s differentiation (14). On the other hand induces
8 the production of TGF- β (39) that control ILCs plasticity (240, 241). Unpublished data suggest
9 BT as a modulator of ILCs and that the regulation of ILCs by BT was associated with
10 maintaining gut homeostasis (242).

11 In conclusion, the adult gut immune system may tolerate self and harmless non-self
12 antigens such as commensal microbiota, while maintaining the ability to recognize and induce
13 a robust proinflammatory response against pathogenic bacteria. Understanding how the gut
14 immune system discriminates between commensals and pathogens is a major challenge,
15 because both express similar microbe-associated molecular patterns. The most abundant
16 commensal bacteria in gut (*Bacteroidetes* and *Firmicutes*) produce SCFAs such BT. BT
17 increase regulatory Gata3⁺ Tregs (243, 244), ROR γ t⁺ Tregs (67), Tr1 cells (107, 155), Bregs
18 (228, 229), M2 macrophages (232-234), tolerogenic dendritic cells (237), and decreases
19 proinflammatory mediators. These findings elucidate pathways through which BT plays an
20 active role in modulating the mucosal immune system to establish a “tolerant” phenotype
21 against beneficial commensal microbiota (Figure 1). Thus, high levels of BT tends to limit
22 immune responses towards the commensals and contribute to the maintenance of microbiota
23 diversity. Upon infection by specific pathogenic bacteria, there is a substantial remodelling of
24 commensal microbiota, resulting in low concentration of BT, allowing for the development of

1 local proinflammatory immune response. The elimination of the pathogen results in the
2 restoration of SCFAs-producing species and the previous host-microbiota equilibrium (181).

3

4 **MICROBIOTA DYSBIOSES AND INFLAMMATORY BOWEL DISEASE**

5 Hippocrates said “all disease begins in the gut” and over 2000 years later we are
6 beginning to appreciate his sentiment. Gut microbiota dysbiosis, i.e. a microbial community
7 imbalance (qualitatively and/or quantitatively), have been suggested to play a role in the
8 development of some proinflammatory diseases, such as IBD (245-247), type 1 diabetes,
9 allergy, asthma, rheumatoid arthritis, and neurological diseases (248, 249). IBD refers to a
10 heterogeneous group of disorders with high rate of incidence in the world and associated with
11 chronic inflammation of the gut (250). Although the cause of IBD remains unknown,
12 considerable progress has been made in the recent years to understand the disease pathogenesis.
13 IBD appears to be triggered by a combination of genetic, environmental and immunologic
14 factors in which an uncontrolled immune response lead to development of chronic
15 inflammation (251). In general, IBD is characterized by an infiltration of large numbers of
16 inflammatory cells in intestine, leukocytosis, abdominal pain, diarrhea and weight loss.
17 However, have the tendency to shift from periods of active disease to periods of little or no
18 disease activity (remission). A large number of environmental factors, such as microbiota
19 composition, infections, diet, drugs, stress, and socioeconomic status have been implicated in
20 the pathogenesis of IBD (251). In addition a large number of genetic susceptibility genes to
21 develop IBD are also involved in immune response to microbiota (e.g. *NOD2*, *TLR4*, *CARD9*,
22 *ATG16L1*, *IL23R* polymorphisms) (251, 252). The most common forms of IBD are Crohn’s
23 disease (CD) primarily affects the small intestine and colon and ulcerative colitis (UC) that is
24 limited to the colon (253). Although CD and UC have been studied together because they share
25 common features (such as symptoms and structural damage), it is now clear that they represent

1 two distinct diseases. For example, the genetic associations are different in both conditions
2 (252) and microbiota has a important role in CD pathogenesis (245, 246), but less relevance in
3 UC (247, 252). CD was designated as a Th1 condition, whereas UC is associated with an
4 exaggerated Th2 mediated response (253). However, this classic view of Th1/Th2 has been
5 challenged because CD and UC have also increased number of Th17 cells and an interplay
6 among Th1, Th2, Th17 and Tregs cells (254). IBD has linked to marked increase in the number
7 of effector immune cells such as proinflammatory TCR $\alpha\beta^+$ IELs (255), macrophages (178),
8 dendritic cells (256, 257), neutrophils (187), mast cells (258), ILCs (259-263), activated lamina
9 propria $\alpha\beta$ T (264, 265) and $\gamma\delta$ T cells (266-268). The increase of effector cells IBD patients
10 may be explained partially by an enhanced influx of these cells into the intestinal wall in
11 response to an increase in cellular adhesion ligands and chemoattractants within the inflamed
12 mucosa (269, 270). Cytokines play a crucial role in wound healing and intestinal inflammation
13 resolution. Therefore, the increase of pro-inflammatory and decrease of anti-inflammatory
14 cytokines that occurs in IBD results in tissue damage, disease progression and limits the
15 resolution of inflammation (254).

16 IBD patients have frequently infections with some opportunistic pathogens suggesting
17 that these bacteria can initiate disease (Table 1) (271). Although these alterations in microbiota
18 composition have long been associated with human IBD pathogenesis, it should be emphasized
19 that the studies discussed so far describe associations and do not prove causation. However, in
20 animal models the acute infection with some pathogens induce microbiota dysbiosis, a dramatic
21 impact on the structure and function of gut immune system, promoting chronic gut
22 inflammation (271-273). An alternative hypothesis suggests that the reduction in the number of
23 tolerogenic bacteria of the phyla *Bacteroidetes* and *Firmicutes* (1, 274-278), particularly the
24 *Firmicutes*, *Clostridium clusters IV* and *XIVa*, e.g. *Faecalibacterium prausnitzii* (279-282), can
25 induce IBD (Figure 2). For example during anaerobic growth *Faecalibacterium prausnitzii*

1 species produce substantial quantities of BT, formate, small amounts of D-lactate and other
2 metabolites that would play a major role in the suppression of inflammation (245). The studies
3 that describe associations of microbiota dysbiosis with IBD pathogenesis do not provide
4 information about the timing of dysbiosis relative to disease onset and should be interpreted
5 with caution particularly with regards to cause-effect relationships. However, IBD patients have
6 reduced numbers of SCFAs-producing bacteria (*Bacteroidetes* and *Firmicutes*) (94) and SCFAs
7 concentration in feces (275, 283-285). In line with this, the administration of probiotic bacteria
8 with the capacity to produce SCFAs has been shown to decrease proinflammatory
9 microenvironment in animal models of IBD (286, 287) and the administration of SCFAs has
10 been used with positive results in the reduction of human colitis severity (288-291). To exert
11 their anti-inflammatory effect in immune cells, SCFAs needs to be absorbed in gut epithelium
12 by two specific transporters, monocarboxylate transporter 1 (MCT1) and/or sodium-coupled
13 monocarboxylate transporter 1 (SMCT1) (139). However, these transporters are downregulated
14 during inflammation and in inflamed mucosa of IBD patients (292-294). The treatment of IECs
15 with proinflammatory mediators (IFN- γ and/or TNF- α) decreased the expression of MCT1 and
16 SMCT1 (292-294), suggesting that the intense inflammatory milieu during IBD causes a
17 decrease in the expression of BT transporters. Therefore, IBD patients showed microbiota
18 dysbiosis, reduced concentration of BT in gut lumen (275, 283, 284) and decreased absorption
19 in gut epithelium (292-294). As described above BT have anti-inflammatory effect in various
20 components of the immune system implicated in gut inflammation: the innate (i.e. intestinal
21 epithelial cells, macrophages, dendritic cells and neutrophils) and adaptive (T and B cells),
22 suggesting that microbiota dysbiosis and reduced gut BT concentration may be involved in IBD
23 pathogenesis. For example, IBD patients showed an increased number of Tregs in inflammatory
24 lesions compared to healthy controls (295-297). Tregs are not able to control the inflammation
25 despite being present in increased numbers. tTregs constitutively expressed Foxp3 (74) and

1 under normal circumstances tTregs cells are very stable and long-lived (298). In contrast, Foxp3
2 expression is less stable in pTregs (59, 299-301). Acetylation of Foxp3 appears to be required
3 for optimal Foxp3 stability and enhanced activity of Tregs (302). BT increases Foxp3
4 acetylation and can enhance pTregs stability and activity (94, 209). Therefore, microbiota
5 dysbiosis and decrease in BT concentration can induce loss of pTregs numbers and/or
6 suppressive activity (74). pTregs have also plasticity to differentiate to other Th types under
7 inflammatory stimuli (59, 299-301). Moreover, is also important to understand if the decrease
8 in BT concentration observed in IBD patients have a role in the acquisition of Tregs
9 proinflammatory capacity. Besides, SCFAs can regulate host systemic immune system (88)
10 and reduced numbers of SCFAs-producing bacteria and SCFAs concentration in gut (275, 283-
11 285) can affect also the development of other systemic proinflammatory diseases such as type
12 1 diabetes (303), lung allergic inflammation (15, 98, 99) and neuroinflammation (89, 100, 101).

13

14 **CONCLUSIONS AND FUTURE PROSPECTS FOR THERAPY**

15 The relationship between microbiota dysbiosis and IBD is complex and dynamic, rather
16 than one of simple cause-effect. Studies attempting to determine whether microbiota dysbiosis
17 is truly causative or merely a consequence of inflammation in humans, have suffered from a
18 number of limitations, making it difficult to have definitive conclusions. However, IBD patients
19 have microbiota dysbiosis with reduced numbers of SCFAs-producing bacteria and reduced
20 concentration of BT in the gut. BT mediates increase in anti-inflammatory cells (e.g. Tregs, Tr1
21 and Bregs) and decreases proinflammatory mediators (e.g. macrophages, dendritic cells
22 neutrophils and effector T cells) in gut, suggesting that microbiota dysbiosis and reduced gut
23 BT concentration are involved in the pathogenesis of IBD. Already in early 1900, commensal
24 microbes have been demonstrated to have beneficial properties in the host, and Ilya Mechnikov
25 had proposed the use of live microbes to maintain gut health and prolong life. Therefore,

1 probiotic supplementation (e.g. orally administered capsules with BT producing bacteria) and
2 modulation of diet (increased prebiotics - fiber) can be one option to treat IBD patients. Another
3 option for manipulation of the gut microbiota involves fecal microbiota transplantation (FMT)
4 from healthy donors to IBD patients (304). Interestingly enough, the richness of donor
5 microbiota with BT-producing bacteria is correlated with successes of FMT (305). Given its
6 anti-inflammatory effect, BT have been proposed to be used in IBD therapy (306). However,
7 the patenting of a natural product has an adverse impact on its clinical development. Some
8 structural analogs of BT were developed, e.g. Tributyrin (307) and AN-9 (308), and some
9 strategies are currently being developed to improve bioavailability of these compounds to use
10 in clinical trials (307, 309).

11

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

13

14 **ACKNOWLEDGMENTS**

15 We thank Antonio Bandeira for the review of the manuscript and discussion.

16

17 **CONFLITCT OF INTEREST**

18 No conflicts of interest are declared by the author(s).

19

20 **LIST OF ABBREVIATIONS**

21 BT - butyrate

22 CD - Crohn’s disease

23 FMT - fecal microbiota transplantation

24 GALT - gut-associated lymphoid tissue

25 GF - germ-free

26 GPR - G-protein-coupled receptor

27 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- κ B - 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

1 REFERENCES

- 2 1. Qin JJ, Li RQ, Raes J, et al. A human gut microbial gene catalogue established by
3 metagenomic sequencing. *Nature*. 2010;464:59-U70
- 4 2. dos Santos VM, Muller M, de Vos WM. Systems biology of the gut: the interplay of food,
5 microbiota and host at the mucosal interface. *Curr Opin Biotech*. 2010;21:539-550
- 6 3. Falony G, Joossens M, Vieira-Silva S, et al. Population-level analysis of gut microbiome
7 variation. *Science*. 2016;352:560-564
- 8 4. Lozupone CA, Stombaugh JI, Gordon JI, et al. Diversity, stability and resilience of the human
9 gut microbiota. *Nature*. 2012;489:220-230
- 10 5. Shade A, Handelsman J. Beyond the Venn diagram: the hunt for a core microbiome. *Environ*
11 *Microbiol*. 2012;14:4-12
- 12 6. Nicholson JK, Holmes E, Kinross J, et al. Host-Gut Microbiota Metabolic Interactions.
13 *Science*. 2012;336:1262-1267
- 14 7. Rautava S, Luoto R, Salminen S, et al. Microbial contact during pregnancy, intestinal
15 colonization and human disease. *Nat Rev Gastroenterol Hepatol*. 2012;9:565-576
- 16 8. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human
17 gut microbiome. *Nature*. 2014;505:559-563
- 18 9. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the
19 acquisition and structure of the initial microbiota across multiple body habitats in newborns. *P*
20 *Natl Acad Sci USA*. 2010;107:11971-11975
- 21 10. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during
22 health and disease (vol 9, pg 313, 2009). *Nat Rev Immunol*. 2009;9:600-600
- 23 11. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, et al. Partial restoration of the
24 microbiota of cesarean-born infants via vaginal microbial transfer. *Nature medicine*.
25 2016;22:250-253
- 26 12. Goodrich JK, Waters JL, Poole AC, et al. Human Genetics Shape the Gut Microbiome.
27 *Cell*. 2014;159:789-799
- 28 13. Chu DM, Ma J, Prince AL, et al. Maturation of the infant microbiome community structure
29 and function across multiple body sites and in relation to mode of delivery. *Nature medicine*.
30 2017;23:314-326
- 31 14. van de Pavert SA, Ferreira M, Domingues RG, et al. Maternal retinoids control type 3 innate
32 lymphoid cells and set the offspring immunity. *Nature*. 2014;508:123-+
- 33 15. Thorburn AN, McKenzie CI, Shen S, et al. Evidence that asthma is a developmental origin
34 disease influenced by maternal diet and bacterial metabolites. *Nat Commun*. 2015;6
- 35 16. Gomez de Agüero M, Ganai-Vonarburg SC, Fuhrer T, et al. The maternal microbiota drives
36 early postnatal innate immune development. *Science*. 2016;351:1296-1302
- 37 17. Koch MA, Reiner GL, Lugo KA, et al. Maternal IgG and IgA Antibodies Dampen Mucosal
38 T Helper Cell Responses in Early Life. *Cell*. 2016;165:827-841
- 39 18. Hooper LV. Bacterial contributions to mammalian gut development. *Trends Microbiol*.
40 2004;12:129-134
- 41 19. Pollard M, Sharon N. Responses of the Peyer's Patches in Germ-Free Mice to Antigenic
42 Stimulation. *Infection and immunity*. 1970;2:96-100
- 43 20. Bouskra D, Brezillon C, Berard M, et al. Lymphoid tissue genesis induced by commensals
44 through NOD1 regulates intestinal homeostasis. *Nature*. 2008;456:507-U534
- 45 21. Bandeira A, Motasantos T, Itohara S, et al. Localization of Gamma-Delta-T-Cells to the
46 Intestinal Epithelium Is Independent of Normal Microbial Colonization. *J Exp Med*.
47 1990;172:239-244
- 48 22. Imaoka A, Matsumoto S, Setoyama H, et al. Proliferative recruitment of intestinal
49 intraepithelial lymphocytes after microbial colonization of germ-free mice. *Eur J Immunol*.
50 1996;26:945-948

- 1 23. Kawaguchi-Miyashita M, Shimizu K, Nanno M, et al. Development and cytolytic function
2 of intestinal intraepithelial T lymphocytes in antigen-minimized mice. *Immunology*.
3 1996;89:268-273
- 4 24. Hooper LV, Littman DR, Macpherson AJ. Interactions Between the Microbiota and the
5 Immune System. *Science*. 2012;336:1268-1273
- 6 25. Zhang D, Chen G, Manwani D, et al. Neutrophil ageing is regulated by the microbiome.
7 *Nature*. 2015
- 8 26. Chung HC, Pamp SJ, Hill JA, et al. Gut Immune Maturation Depends on Colonization with
9 a Host-Specific Microbiota. *Cell*. 2012;149:1578-1593
- 10 27. Sanos SL, Bui VL, Mortha A, et al. ROR γ and commensal microflora are required
11 for the differentiation of mucosal interleukin 22-producing NKp46⁺ cells. *Nat Immunol*.
12 2009;10:83-91
- 13 28. Satoh-Takayama N, Voshenrich CAJ, Lesjean-Pottier S, et al. Microbial Flora Drives
14 Interleukin 22 Production in Intestinal NKp46(+) Cells that Provide Innate Mucosal Immune
15 Defense. *Immunity*. 2008;29:958-970
- 16 29. Martin E, Treiner E, Duban L, et al. Stepwise Development of MAIT Cells in Mouse and
17 Human. *Plos Biol*. 2009;7:525-536
- 18 30. Treiner E, Duban L, Bahram S, et al. Selection of evolutionarily conserved mucosal-
19 associated invariant T cells by MR1. *Nature*. 2003;422:164-169
- 20 31. Atarashi K, Nishimura J, Shima T, et al. ATP drives lamina propria T(H)17 cell
21 differentiation. *Nature*. 2008;455:808-812
- 22 32. Ivanov, II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of
23 IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe*.
24 2008;4:337-349
- 25 33. Duan J, Chung H, Troy E, et al. Microbial colonization drives expansion of IL-1 receptor
26 1-expressing and IL-17-producing gamma/delta T cells. *Cell host & microbe*. 2010;7:140-150
- 27 34. Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous
28 *Clostridium* species. *Science*. 2011;331:337-341
- 29 35. Hapfelmeier S, Lawson MA, Slack E, et al. Reversible microbial colonization of germ-free
30 mice reveals the dynamics of IgA immune responses. *Science*. 2010;328:1705-1709
- 31 36. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of
32 symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005;122:107-118
- 33 37. Ivanov II, Atarashi K, Manel N, et al. Induction of Intestinal Th17 Cells by Segmented
34 Filamentous Bacteria. *Cell*. 2009;139:485-498
- 35 38. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, et al. The Key Role of Segmented
36 Filamentous Bacteria in the Coordinated Maturation of Gut Helper T Cell Responses.
37 *Immunity*. 2009;31:677-689
- 38 39. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of
39 *Clostridia* strains from the human microbiota. *Nature*. 2013;500:232-236
- 40 40. Schirmer M, Smeekens SP, Vlamakis H, et al. Linking the Human Gut Microbiome to
41 Inflammatory Cytokine Production Capacity (vol 167, pg 1125, 2016). *Cell*. 2016;167:1897-
42 1897
- 43 41. Liston A. The cellular composition of the human immune system is shaped by age and
44 cohabitation. *Journal of Immunology*. 2016;196
- 45 42. Yatsunencko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and
46 geography. *Nature*. 2012;486:222-+
- 47 43. Lax S, Smith DP, Hampton-Marcell J, et al. Longitudinal analysis of microbial interaction
48 between humans and the indoor environment. *Science*. 2014;345:1048-1052
- 49 44. Kort R, Caspers M, van de Graaf A, et al. Shaping the oral microbiota through intimate
50 kissing. *Microbiome*. 2014;2

- 1 45. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against
2 respiratory tract influenza A virus infection. *P Natl Acad Sci USA*. 2011;108:5354-5359
- 3 46. Abt MC, Osborne LC, Monticelli LA, et al. Commensal Bacteria Calibrate the Activation
4 Threshold of Innate Antiviral Immunity. *Immunity*. 2012;37:158-170
- 5 47. Wardwell LH, Huttenhower C, Garrett WS. Current concepts of the intestinal microbiota
6 and the pathogenesis of infection. *Curr Infect Dis Rep.* 2011;13:28-34
- 7 48. Rivera-Chavez F, Zhang LF, Faber F, et al. Depletion of Butyrate-Producing Clostridia
8 from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host*
9 *Microbe*. 2016;19:443-454
- 10 49. Sonnenberg GF, Monticelli LA, Alenghat T, et al. Innate Lymphoid Cells Promote
11 Anatomical Containment of Lymphoid-Resident Commensal Bacteria. *Science*.
12 2012;336:1321-1325
- 13 50. Obata T, Goto Y, Kunisawa J, et al. Indigenous opportunistic bacteria inhabit mammalian
14 gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *P Natl Acad*
15 *Sci USA*. 2010;107:7419-7424
- 16 51. Fung TC, Bessman NJ, Hepworth MR, et al. Lymphoid-Tissue-Resident Commensal
17 Bacteria Promote Members of the IL-10 Cytokine Family to Establish Mutualism. *Immunity*.
18 2016;44:634-646
- 19 52. Cheroutre H, Madakamutil L. Acquired and natural memory T cells join forces at the
20 mucosal front line. *Nat Rev Immunol*. 2004;4:290-300
- 21 53. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial
22 lymphocytes. *Nat Rev Immunol*. 2011;11:445-456
- 23 54. Rocha B, Vassalli P, Guy-Grand D. The V beta repertoire of mouse gut homodimeric alpha
24 CD8+ intraepithelial T cell receptor alpha/beta + lymphocytes reveals a major extrathymic
25 pathway of T cell differentiation. *J Exp Med*. 1991;173:483-486
- 26 55. Umesaki Y, Setoyama H, Matsumoto S, et al. Expansion of Alpha-Beta T-Cell Receptor-
27 Bearing Intestinal Intraepithelial Lymphocytes after Microbial Colonization in Germ-Free
28 Mice and Its Independence from Thymus. *Immunology*. 1993;79:32-37
- 29 56. Umesaki Y, Okada Y, Matsumoto S, et al. Segmented Filamentous Bacteria Are Indigenous
30 Intestinal Bacteria That Activate Intraepithelial Lymphocytes and Induce Mhc Class-II
31 Molecules and Fucosyl Asialo Gm1 Glycolipids on the Small-Intestinal Epithelial-Cells in the
32 Ex-Germ-Free Mouse. *Microbiol Immunol*. 1995;39:555-562
- 33 57. Regnault A, Levraud JP, Lim A, et al. The expansion and selection of T cell receptor alpha
34 beta intestinal intraepithelial T cell clones. *Eur J Immunol*. 1996;26:914-921
- 35 58. Helgeland L, Dissen E, Dai KZ, et al. Microbial colonization induces oligoclonal
36 expansions of intraepithelial CD8 T cells in the gut. *Eur J Immunol*. 2004;34:3389-3400
- 37 59. Yadav M, Stephan S, Bluestone JA. Peripherally induced Tregs - role in immune
38 homeostasis and autoimmunity. *Front Immunol*. 2013;4
- 39 60. Resendiz-Albor AA, Esquivel R, Lopez-Revilla R, et al. Striking phenotypic and functional
40 differences in lamina propria lymphocytes from the large and small intestine of mice. *Life*
41 *sciences*. 2005;76:2783-2803
- 42 61. Moon C, Baldridge MT, Wallace MA, et al. Vertically transmitted faecal IgA levels
43 determine extra-chromosomal phenotypic variation. *Nature*. 2015;521:90-U209
- 44 62. Fagarasan S, Kinoshita K, Muramatsu M, et al. In situ class switching and differentiation
45 to IgA-producing cells in the gut lamina propria. *Nature*. 2001;413:639-643
- 46 63. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of
47 intestinal mucosal IgA responses to commensal bacteria. *Science*. 2000;288:2222-+
- 48 64. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying
49 commensal bacteria. *Science*. 2004;303:1662-1665

- 1 65. Craig SW, Cebra JJ. Peyer's patches: an enriched source of precursors for IgA-producing
2 immunocytes in the rabbit. *J Exp Med.* 1971;134:188-200
- 3 66. Maloy KJ, Kullberg MC. IL-23 and Th17 cytokines in intestinal homeostasis. *Mucosal*
4 *immunology.* 2008;1:339-349
- 5 67. Ohnmacht C, Park JH, Cording S, et al. The microbiota regulates type 2 immunity through
6 RORgammat+ T cells. *Science.* 2015;349:989-93
- 7 68. Tanoue T, Atarashi K, Honda K. Development and maintenance of intestinal regulatory T
8 cells. *Nat Rev Immunol.* 2016; 6:295-309
- 9 69. Sefik E, Geva-Zatorsky N, Oh S, et al. Individual intestinal symbionts induce a distinct
10 population of RORgamma+ regulatory T cells. *Science.* 2015; 49:993-7
- 11 70. Lathrop SK, Bloom SM, Rao SM, et al. Peripheral education of the immune system by
12 colonic commensal microbiota. *Nature.* 2011;478:250-254
- 13 71. Bacher P, Kniemeyer O, Schonbrunn A, et al. Antigen-specific expansion of human
14 regulatory T cells as a major tolerance mechanism against mucosal fungi. *Mucosal*
15 *immunology.* 2014;7:916-928
- 16 72. Yang Y, Torchinsky MB, Gobert M, et al. Focused specificity of intestinal TH17 cells
17 towards commensal bacterial antigens. *Nature.* 2014;510:152-156
- 18 73. Nishio J, Baba M, Atarashi K, et al. Requirement of full TCR repertoire for regulatory T
19 cells to maintain intestinal homeostasis. *Proc Natl Acad Sci U S A.* 2015;112:12770-5.
- 20 74. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program
21 in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol.* 2007;8:277-
22 284
- 23 75. Cebula A, Seweryn M, Rempala GA, et al. Thymus-derived regulatory T cells contribute to
24 tolerance to commensal microbiota. *Nature.* 2013;497:258-+
- 25 76. Goncalves P, Di Santo JP. An Intestinal Inflammasome - The ILC3-Cytokine Tango. *Trends*
26 *Mol Med.* 2. 2016;22:269-271
- 27 77. Eberl G, Colonna M, Di Santo JP, et al. Innate lymphoid cells: A new paradigm in
28 immunology. *Science.* 2015;348:879-+
- 29 78. Lindemans CA, Calafiore M, Mertelsmann AM, et al. Interleukin-22 promotes intestinal-
30 stem-cell-mediated epithelial regeneration. *Nature.* 2015;528:560-4
- 31 79. Goto Y, Obata T, Kunisawa J, et al. Innate lymphoid cells regulate intestinal epithelial cell
32 glycosylation. *Science.* 2014;345:1254009
- 33 80. Pickard JM, Maurice CF, Kinnebrew MA, et al. Rapid fucosylation of intestinal epithelium
34 sustains host-commensal symbiosis in sickness. *Nature.* 2014;514:638-641
- 35 81. Kumar P, Monin L, Castillo P, et al. Intestinal Interleukin-17 Receptor Signaling Mediates
36 Reciprocal Control of the Gut Microbiota and Autoimmune Inflammation. *Immunity.*
37 2016;44:659-671
- 38 82. Hepworth MR, Fung TC, Masur SH, et al. Immune tolerance. Group 3 innate lymphoid
39 cells mediate intestinal selection of commensal bacteria-specific CD4(+) T cells. *Science.*
40 2015;348:1031-1035
- 41 83. Mortha A, Chudnovskiy A, Hashimoto D, et al. Microbiota-Dependent Crosstalk Between
42 Macrophages and ILC3 Promotes Intestinal Homeostasis. *Science.* 2014;343:1477-+
- 43 84. Kruglov AA, Grivennikov SI, Kuprash DV, et al. Nonredundant function of soluble
44 LTalpha3 produced by innate lymphoid cells in intestinal homeostasis. *Science.*
45 2013;342:1243-1246
- 46 85. Sonnenberg GF, Monticelli LA, Elloso MM, et al. CD4(+) Lymphoid Tissue-Inducer Cells
47 Promote Innate Immunity in the Gut. *Immunity.* 2011;34:122-134
- 48 86. Boettcher S, Manz MG. Sensing and translation of pathogen signals into demand-adapted
49 myelopoiesis. *Curr Opin Hematol.* 2016;23:5-10

- 1 87. Khosravi A, Yanez A, Price JG, et al. Gut Microbiota Promote Hematopoiesis to Control
2 Bacterial Infection. *Cell Host Microbe*. 2014;15:374-381
- 3 88. Beura LK, Hamilton SE, Bi K, et al. Normalizing the environment recapitulates adult human
4 immune traits in laboratory mice. *Nature*. 2016; 532:512-6
- 5 89. Benakis C, Brea D, Caballero S, et al. Commensal microbiota affects ischemic stroke
6 outcome by regulating intestinal gammadelta T cells. *Nature medicine*. 2016;22:516-523
- 7 90. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the
8 development of Type 1 diabetes. *Nature*. 2008;455:1109-U1110
- 9 91. Kau AL, Ahern PP, Griffin NW, et al. Human nutrition, the gut microbiome and the immune
10 system. *Nature*. 2011;474:327-336
- 11 92. Kjer-Nielsen L, Patel O, Corbett AJ, et al. MR1 presents microbial vitamin B metabolites
12 to MAIT cells. *Nature*. 2012;491:717-723
- 13 93. An DD, Oh SF, Olszak T, et al. Sphingolipids from a Symbiotic Microbe Regulate
14 Homeostasis of Host Intestinal Natural Killer T Cells. *Cell*. 2014;156:123-133
- 15 94. Arpaia N, Campbell C, Fan XY, et al. Metabolites produced by commensal bacteria promote
16 peripheral regulatory T-cell generation. *Nature*. 2013;504:451-+
- 17 95. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing
18 bacteria from the human large intestine. *FEMS Microbiol Lett*. 2009;294:1-8
- 19 96. Cavaglieri CR, Nishiyama A, Fernandes LC, et al. Differential effects of short-chain fatty
20 acids on proliferation and production of pro- and anti-inflammatory cytokines by cultured
21 lymphocytes. *Life sciences*. 2003;73:1683-1690
- 22 97. Segain JP, de la Bletiere DR, Bourreille A, et al. Butyrate inhibits inflammatory responses
23 through NF kappa B inhibition: implications for Crohn's disease. *Gut*. 2000;47:397-403
- 24 98. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber
25 influences allergic airway disease and hematopoiesis. *Nature medicine*. 2014;20:159-166
- 26 99. Zhang Z, Shi L, Pang W, et al. Dietary Fiber Intake Regulates Intestinal Microflora and
27 Inhibits Ovalbumin-Induced Allergic Airway Inflammation in a Mouse Model. *PloS one*.
28 2016;11:e0147778
- 29 100. Erny D, de Angelis ALH, Jaitin D, et al. Host microbiota constantly control maturation
30 and function of microglia in the CNS. *Nat Neurosci*. 2015;18:965-+
- 31 101. Sherry CL, Kim SS, Dilger RN, et al. Sickness behavior induced by endotoxin can be
32 mitigated by the dietary soluble fiber, pectin, through up-regulation of IL-4 and Th2
33 polarization. *Brain Behav Immun*. 2010;24:631-640
- 34 102. Laukoetter MG, Bruewer M, Nusrat A. Regulation of the intestinal epithelial barrier by
35 the apical junctional complex. *Curr Opin Gastroenterol*. 2006;22:85-89
- 36 103. Finnie IA, Dwarakanath AD, Taylor BA, et al. Colonic Mucin Synthesis Is Increased by
37 Sodium-Butyrate. *Gut*. 1995;36:93-99
- 38 104. Raqib R, Sarker P, Bergman P, et al. Improved outcome in shigellosis associated with
39 butyrate induction of an endogenous peptide antibiotic. *P Natl Acad Sci USA*. 2006;103:9178-
40 9183
- 41 105. Xiong H, Guo B, Gan Z, et al. Butyrate upregulates endogenous host defense peptides to
42 enhance disease resistance in piglets via histone deacetylase inhibition. *Sci Rep*. 2016;6:27070
- 43 106. Macia L, Tan J, Vieira AT, et al. Metabolite-sensing receptors GPR43 and GPR109A
44 facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat*
45 *Commun*. 2015;6:6734.
- 46 107. Singh N, Gurav A, Sivaprakasam S, et al. Activation of Gpr109a, Receptor for Niacin and
47 the Commensal Metabolite Butyrate, Suppresses Colonic Inflammation and Carcinogenesis.
48 *Immunity*. 2014;40:128-139

- 1 108. Levy M, Thaiss CA, Zeevi D, et al. Microbiota-Modulated Metabolites Shape the
2 Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell*.
3 2015;163:1428-1443
- 4 109. Kaiko GE, Ryu SH, Koues OI, et al. The Colonic Crypt Protects Stem Cells from
5 Microbiota-Derived Metabolites. *Cell*. 2016;167:1137.
- 6 110. Kelly CJ, Zheng L, Campbell EL, et al. Crosstalk between Microbiota-Derived Short-
7 Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host*
8 *Microbe*. 2015;17:662-671
- 9 111. Goncalves P, Martel F. Butyrate and colorectal cancer: the role of butyrate transport. *Curr*
10 *Drug Metab*. 2013;14:994-1008
- 11 112. Murakoshi S, Fukatsu K, Omata J, et al. Effects of Adding Butyric Acid to PN on Gut-
12 Associated Lymphoid Tissue and Mucosal Immunoglobulin A Levels. *Jpen-Parenter Enter*.
13 2011;35:465-472
- 14 113. Xu WF, He B, Chiu A, et al. Epithelial cells trigger frontline immunoglobulin class
15 switching through a pathway regulated by the inhibitor SLPI. *Nat Immunol*. 2007;8:294-303
- 16 114. Ishikawa T, Nanjo F. Dietary Cycloinooligosaccharides Enhance Intestinal
17 Immunoglobulin A Production in Mice. *Biosci Biotech Bioch*. 2009;73:677-682
- 18 115. Kim M, Qie YQ, Park J, et al. Gut Microbial Metabolites Fuel Host Antibody Responses.
19 *Cell Host Microbe*. 2016;20:202-214
- 20 116. Suzuki K, Meek B, Doi Y, et al. Aberrant expansion of segmented filamentous bacteria in
21 IgA-deficient gut. *P Natl Acad Sci USA*. 2004;101:1981-1986
- 22 117. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune
23 homeostasis. *Nat Rev Immunol*. 2014;14:141-153
- 24 118. Iliiev ID, Mileti E, Matteoli G, et al. Intestinal epithelial cells promote colitis-protective
25 regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal immunology*.
26 2009;2:340-350
- 27 119. Iliiev ID, Spadoni I, Mileti E, et al. Human intestinal epithelial cells promote the
28 differentiation of tolerogenic dendritic cells. *Gut*. 2009;58:1481-1489
- 29 120. Colgan SP, Hershberg RM, Furuta GT, et al. Ligation of intestinal epithelial CD1d induces
30 bioactive IL-10: Critical role of the cytoplasmic tail in autocrine signaling. *P Natl Acad Sci*
31 *USA*. 1999;96:13938-13943
- 32 121. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial
33 recognition shapes intestinal function. *Nat Rev Immunol*. 2010;10:131-143
- 34 122. Kumar H, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys*
35 *Res Commun*. 2009;388:621-625
- 36 123. Yang SK, Eckmann L, Panja A, et al. Differential and regulated expression of C-X-C, C-
37 C, and C-chemokines by human colon epithelial cells. *Gastroenterology*. 1997;113:1214-1223
- 38 124. Lee SK, Kim TI, Kim YK, et al. Cellular differentiation-induced attenuation of LPS
39 response in HT-29 cells is related to the down-regulation of TLR4 expression. *Biochem*
40 *Biophys Res Commun*. 2005;337:457-463
- 41 125. Liu B, Qian J, Wang Q, et al. Butyrate Protects Rat Liver against Total Hepatic Ischemia
42 Reperfusion Injury with Bowel Congestion. *PloS one*. 2014;9:e106184
- 43 126. Lee JW, Wang P, Kattah MG, et al. Differential Regulation of Chemokines by IL-17 in
44 Colonic Epithelial Cells. *Journal of Immunology*. 2008;181:6536-6545
- 45 127. Asarat M, Vasiljevic T, Apostolopoulos V, et al. Short-Chain Fatty Acids Regulate
46 Secretion of IL-8 from Human Intestinal Epithelial Cell Lines in vitro. *Immunol Invest*.
47 2015;44:678-693
- 48 128. Blais M, Seidman EG, Asselin C. Dual effect of butyrate on IL-1beta--mediated intestinal
49 epithelial cell inflammatory response. *DNA Cell Biol*. 2007;26:133-147

- 1 129. Ganapathy V, Thangaraju M, Cresci GA, et al. GPR109A Is a G-protein-Coupled Receptor
2 for the Bacterial Fermentation Product Butyrate and Functions as a Tumor Suppressor in Colon.
3 *Cancer Res.* 2009;69:2826-2832
- 4 130. Brown AJ, Goldsworthy SM, Barnes AA, et al. The orphan G protein-coupled receptors
5 GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol*
6 *Chem.* 2003;278:11312-11319
- 7 131. Le Poul E, Loison C, Struyf S, et al. Functional characterization of human receptors for
8 short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem.*
9 2003;278:25481-25489
- 10 132. Ulven T. Short-chain free fatty acid receptors FFA2/GPR43 and FFA3/GPR41 as new
11 potential therapeutic targets. *Front Endocrinol (Lausanne).* 2012;3:111
- 12 133. Yousefi S, Cooper PR, Mueck B, et al. cDNA representational difference analysis of
13 human neutrophils stimulated by GM-CSF. *Biochem Biophys Res Commun.* 2000;277:401-
14 409
- 15 134. Schaub A, Futterer A, Pfeffer K. PUMA-G, an IFN-gamma-inducible gene in
16 macrophages is a novel member of the seven transmembrane spanning receptor superfamily.
17 *Eur J Immunol.* 2001;31:3714-3725
- 18 135. Wu ST, Li RW, Li WZ, et al. Transcriptome Characterization by RNA-seq Unravels the
19 Mechanisms of Butyrate-Induced Epigenomic Regulation in Bovine Cells. *PLoS One.* 2012;7
20 136. Maslowski KM, Vieira AT, Ng A, et al. Regulation of inflammatory responses by gut
21 microbiota and chemoattractant receptor GPR43. *Nature.* 2009;461:1282-1286
- 22 137. Xiong YM, Miyamoto N, Shibata K, et al. Short-chain fatty acids stimulate leptin
23 production in adipocytes through the G protein-coupled receptor GPR41. *P Natl Acad Sci USA.*
24 2004;101:1045-1050
- 25 138. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty
26 acids, regulate colonic Treg cell homeostasis. *Science.* 2013;341:569-573
- 27 139. Hamer HM, Jonkers D, Venema K, et al. Review article: the role of butyrate on colonic
28 function. *Aliment Pharm Therap.* 2008;27:104-119
- 29 140. Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr.* 2003;133:2485S-
30 2493S
- 31 141. Daroqui MC, Augenlicht LH. Transcriptional attenuation in colon carcinoma cells in
32 response to butyrate. *Cancer Prev Res (Phila).* 2010;3:1292-1302
- 33 142. Wong JM, de Souza R, Kendall CW, et al. Colonic health: fermentation and short chain
34 fatty acids. *J Clin Gastroenterol.* 2006;40:235-243
- 35 143. Sekhavat A, Sun JM, Davie JR. Competitive inhibition of histone deacetylase activity by
36 trichostatin A and butyrate. *Biochem Cell Biol.* 2007;85:751-758
- 37 144. Thangaraju M, Carswell KN, Prasad PD, et al. Colon cancer cells maintain low levels of
38 pyruvate to avoid cell death caused by inhibition of HDAC1/HDAC3. *Biochemical Journal.*
39 2009;417:379-389
- 40 145. Luhrs H, Gerke T, Muller JG, et al. Butyrate inhibits NF-kappaB activation in lamina
41 propria macrophages of patients with ulcerative colitis. *Scand J Gastroenterol.* 2002;37:458-
42 466
- 43 146. Felice C, Lewis A, Armuzzi A, et al. Review article: selective histone deacetylase isoforms
44 as potential therapeutic targets in inflammatory bowel diseases. *Aliment Pharm Ther.*
45 2015;41:26-38
- 46 147. Kinoshita M, Suzuki Y, Saito Y. Butyrate reduces colonic paracellular permeability by
47 enhancing PPARgamma activation. *Biochem Biophys Res Commun.* 2002;293:827-831
- 48 148. Ricote M, Li AC, Willson TM, et al. The peroxisome proliferator-activated receptor-
49 gamma is a negative regulator of macrophage activation. *Nature.* 1998;391:79-82

- 1 149. White NR, Mulligan P, King PJ, et al. Sodium butyrate-mediated Sp3 acetylation represses
2 human insulin-like growth factor binding protein-3 expression in intestinal epithelial cells. *J*
3 *Pediatr Gastr Nutr.* 2006;42:134-141
- 4 150. Ranganna K, Mathew OP, Yatsu FM. Butyrate, an HDAC inhibitor, stimulates interplay
5 between different posttranslational modifications of histone H3 and differently alters G1-
6 specific cell cycle proteins in vascular smooth muscle cells. *Biomed Pharmacother.*
7 2010;64:733-740
- 8 151. Mizzen CA, Pesavento JJ, Yang H, et al. Certain and progressive methylation of histone
9 H4 at lysine 20 during the cell cycle. *Mol Cell Biol.* 2008;28:468-486
- 10 152. de Haan JB, Gevers W, Parker MI. Effects of sodium butyrate on the synthesis and
11 methylation of DNA in normal cells and their transformed counterparts. *Cancer Res.*
12 1986;46:713-716
- 13 153. Donohoe DR, Collins LB, Wali A, et al. The Warburg Effect Dictates the Mechanism of
14 Butyrate-Mediated Histone Acetylation and Cell Proliferation. *Mol Cell.* 2012;48:612-626
- 15 154. Donohoe DR, Garge N, Zhang XX, et al. The Microbiome and Butyrate Regulate Energy
16 Metabolism and Autophagy in the Mammalian Colon. *Cell Metab.* 2011;13:517-526
- 17 155. Park J, Kim M, Kang SG, et al. Short-chain fatty acids induce both effector and regulatory
18 T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway.
19 *Mucosal immunology.* 2014; 8:80-93
- 20 156. Delgoffe GM, Pollizzi KN, Waickman AT, et al. The kinase mTOR regulates the
21 differentiation of helper T cells through the selective activation of signaling by mTORC1 and
22 mTORC2. *Nat Immunol.* 2011;12:295-U117
- 23 157. Pollizzi KN, Patel CH, Sun IH, et al. mTORC1 and mTORC2 selectively regulate CD8(+)
24 T cell differentiation. *J Clin Invest.* 2015;125:2090-2108
- 25 158. Millard AL, Mertes PM, Ittelet D, et al. Butyrate affects differentiation, maturation and
26 function of human monocyte-derived dendritic cells and macrophages. *Clin Exp Immunol.*
27 2002;130:245-255
- 28 159. Singh N, Thangaraju M, Prasad PD, et al. Blockade of dendritic cell development by
29 bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-
30 dependent inhibition of histone deacetylases. *J Biol Chem.* 2010;285:27601-27608
- 31 160. Zimmerman MA, Singh N, Martin PM, et al. Butyrate suppresses colonic inflammation
32 through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. *Am J*
33 *Physiol-Gastr L.* 2012;302:G1405-G1415
- 34 161. Nascimento CR, Freire-de-Lima CG, de Oliveira AD, et al. The short chain fatty acid
35 sodium butyrate regulates the induction of CD1a in developing dendritic cells. *Immunobiology.*
36 2011;216:275-284
- 37 162. Nencioni A, Beck J, Werth D, et al. Histone deacetylase inhibitors affect dendritic cell
38 differentiation and immunogenicity. *Clin Cancer Res.* 2007;13:3933-3941
- 39 163. Bosisio D, Vulcano M, Del Prete A, et al. Blocking TH17-polarizing cytokines by histone
40 deacetylase inhibitors in vitro and in vivo. *J Leukoc Biol.* 2008;84:1540-1548
- 41 164. Yamaguchi T, Cubizolles F, Zhang Y, et al. Histone deacetylases 1 and 2 act in concert to
42 promote the G1-to-S progression. *Genes Dev.* 2010;24:455-469
- 43 165. Yamamoto I, Adachi T, Kishiro Y, et al. Interleukin-2-dependent augmentation of the anti-
44 TNP antibody production by sodium butyrate in cultured murine splenic B cells. *Int J*
45 *Immunopharmacol.* 1997;19:347-354
- 46 166. Newman JD, Eckardt GS, Boyd A, et al. Induction of the Insulin-Receptor and Other
47 Differentiation Markers by Sodium-Butyrate in the Burkitt-Lymphoma Cell, Raji. *Biochem*
48 *Biophys Res Commun.* 1989;161:101-106

- 1 167. Zapolska-Downar D, Siennicka A, Kaczmarczyk M, et al. Butyrate inhibits cytokine-
2 induced VCAM-1 and ICAM-1 expression in cultured endothelial cells: the role of NF-kappaB
3 and PPARalpha. *J Nutr Biochem.* 2004;15:220-228
- 4 168. Aguilar EC, Leonel AJ, Teixeira LG, et al. Butyrate impairs atherogenesis by reducing
5 plaque inflammation and vulnerability and decreasing NFkappaB activation. *Nutr Metab*
6 *Cardiovasc Dis.* 2014;24:606-613
- 7 169. Maa MC, Chang MY, Hsieh MY, et al. Butyrate reduced lipopolysaccharide-mediated
8 macrophage migration by suppression of Src enhancement and focal adhesion kinase activity.
9 *J Nutr Biochem.* 2010;21:1186-1192
- 10 170. Kolaczowska E, Kubes P. Neutrophil recruitment and function in health and
11 inflammation. *Nat Rev Immunol.* 2013;13:159-175
- 12 171. Nastasi C, Candela M, Bonefeld CM, et al. The effect of short-chain fatty acids on human
13 monocyte-derived dendritic cells. *Scientific reports.* 2015;5:16148
- 14 172. Zhang L, Jin SW, Wang CD, et al. Histone Deacetylase Inhibitors Attenuate Acute Lung
15 Injury During Cecal Ligation and Puncture-Induced Polymicrobial Sepsis. *World J Surg.*
16 2010;34:1676-1683
- 17 173. Vinolo MA, Rodrigues HG, Hatanaka E, et al. Suppressive effect of short-chain fatty acids
18 on production of proinflammatory mediators by neutrophils. *J Nutr Biochem.* 2011;22:849-855
- 19 174. Tao R, de Zoeten EF, Ozkaynak E, et al. Deacetylase inhibition promotes the generation
20 and function of regulatory T cells. *Nature medicine.* 2007;13:1299-1307
- 21 175. Kim SV, Xiang WKV, Kwak C, et al. GPR15-Mediated Homing Controls Immune
22 Homeostasis in the Large Intestine Mucosa. *Science.* 2013;340:1456-1459
- 23 176. Nguyen LP, Pan JL, Dinh TT, et al. Role and species-specific expression of colon T cell
24 homing receptor GPR15 in colitis. *Nat Immunol.* 2015;16:207-+
- 25 177. Zaiss MM, Rapin A, Lebon L, et al. The Intestinal Microbiota Contributes to the Ability
26 of Helminths to Modulate Allergic Inflammation. *Immunity.* 2015;43:998-1010
- 27 178. Zigmund E, Jung S. Intestinal macrophages: well educated exceptions from the rule.
28 *Trends Immunol.* 2013;34:162-168
- 29 179. Nicodeme E, Jeffrey KL, Schaefer U, et al. Suppression of inflammation by a synthetic
30 histone mimic. *Nature.* 2010;468:1119-1123
- 31 180. Leoni F, Zaliani A, Bertolini G, et al. The antitumor histone deacetylase inhibitor
32 suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of
33 cytokines. *Proc Natl Acad Sci U S A.* 2002;99:2995-3000
- 34 181. Chang PV, Hao L, Offermanns S, et al. The microbial metabolite butyrate regulates
35 intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci U S A.*
36 2014;111:2247-2252
- 37 182. Chakravorty D, Koide N, Kato Y, et al. The inhibitory action of butyrate on
38 lipopolysaccharide-induced nitric oxide production in RAW 264.7 murine macrophage cells. *J*
39 *Endotoxin Res.* 2000;6:243-247
- 40 183. Ramos MG, Rabelo FLA, Duarte T, et al. Butyrate induces apoptosis in murine
41 macrophages via caspase-3, but independent of autocrine synthesis of tumor necrosis factor and
42 nitric oxide. *Braz J Med Biol Res.* 2002;35:161-173
- 43 184. Brogdon JL, Xu Y, Szabo SJ, et al. Histone deacetylase activities are required for innate
44 immune cell control of Th1 but not Th2 effector cell function. *Blood.* 2007;109:1123-1130
- 45 185. Roger T, Lugrin J, Le Roy D, et al. Histone deacetylase inhibitors impair innate immune
46 responses to Toll-like receptor agonists and to infection. *Blood.* 2011;117:1205-1217
- 47 186. Backdahl L, Bushell A, Beck S. Inflammatory signalling as mediator of epigenetic
48 modulation in tissue-specific chronic inflammation. *Int J Biochem Cell B.* 2009;41:176-184

- 1 187. Takeda K, Clausen BE, Kaisho T, et al. Enhanced Th1 activity and development of chronic
2 enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity*. 1999;10:39-
3 49
- 4 188. Vinolo MAR, Ferguson GJ, Kulkarni S, et al. SCFAs Induce Mouse Neutrophil
5 Chemotaxis through the GPR43 Receptor. *PloS one*. 2011;6
- 6 189. Vinolo MA, Hatanaka E, Lambertucci RH, et al. Effects of short chain fatty acids on
7 effector mechanisms of neutrophils. *Cell Biochem Funct*. 2009;27:48-55
- 8 190. Mills SW, Montgomery SH, Morck DW. Evaluation of the effects of short-chain fatty
9 acids and extracellular pH on bovine neutrophil function in vitro. *Am J Vet Res*. 2006;67:1901-
10 1907
- 11 191. Eftimiadi C, Tonetti M, Cavallero A, et al. Short-chain fatty acids produced by anaerobic
12 bacteria inhibit phagocytosis by human lung phagocytes. *J Infect Dis*. 1990;161:138-142
- 13 192. Mirmonsef P, Zariffard MR, Gilbert D, et al. Short-chain fatty acids induce pro-
14 inflammatory cytokine production alone and in combination with toll-like receptor ligands. *Am*
15 *J Reprod Immunol*. 2012;67:391-400
- 16 193. Liu Q, Shimoyama T, Suzuki K, et al. Effect of sodium butyrate on reactive oxygen species
17 generation by human neutrophils. *Scand J Gastroenterol*. 2001;36:744-750
- 18 194. Aoyama M, Kotani J, Usami M. Butyrate and propionate induced activated or non-
19 activated neutrophil apoptosis via HDAC inhibitor activity but without activating GPR-
20 41/GPR-43 pathways. *Nutrition*. 2010;26:653-661
- 21 195. Kostylina G, Simon D, Fey MF, et al. Neutrophil apoptosis mediated by nicotinic acid
22 receptors (GPR109A). *Cell Death Differ*. 2008;15:134-142
- 23 196. Zhang H, Du M, Yang Q, et al. Butyrate suppresses murine mast cell proliferation and
24 cytokine production through inhibiting histone deacetylase. *J Nutr Biochem*. 2016;27:299-306
- 25 197. Kyner D, Zalos P, Christman J, et al. Effect of sodium butyrate on lymphocyte activation.
26 *J Exp Med*. 1976;144:1674-1678
- 27 198. Dagtas AS, Edens RE, Gilbert KM. Histone deacetylase inhibitor uses p21(Cip1) to
28 maintain anergy in CD4+ T cells. *Int Immunopharmacol*. 2009;9:1289-1297
- 29 199. Stenzel KH, Schwartz R, Rubin AL, et al. Chemical Inducers of Differentiation in Friend-
30 Leukemia Cells Inhibit Lymphocyte Mitogenesis. *Nature*. 1980;285:106-108
- 31 200. Gilbert KM, Weigle WO. Th1 cell anergy and blockade in G1a phase of the cell cycle. *J*
32 *Immunol*. 1993;151:1245-1254
- 33 201. Moreira JM, Scheipers P, Sorensen P. The histone deacetylase inhibitor Trichostatin A
34 modulates CD4+ T cell responses. *Bmc Cancer*. 2003;3:30
- 35 202. Kurita-Ochiai T, Hashizume T, Yonezawa H, et al. Characterization of the effects of
36 butyric acid on cell proliferation, cell cycle distribution and apoptosis. *FEMS Immunol Med*
37 *Microbiol*. 2006;47:67-74
- 38 203. Bohmig GA, Krieger PM, Saemann MD, et al. n-butyrate downregulates the stimulatory
39 function of peripheral blood-derived antigen-presenting cells: a potential mechanism for
40 modulating T-cell responses by short-chain fatty acids. *Immunology*. 1997;92:234-243
- 41 204. Michalek RD, Gerriets VA, Jacobs SR, et al. Cutting Edge: Distinct Glycolytic and Lipid
42 Oxidative Metabolic Programs Are Essential for Effector and Regulatory CD4(+) T Cell
43 Subsets. *Journal of Immunology*. 2011;186:3299-3303
- 44 205. Fischer K, Hoffmann P, Voelkl S, et al. Inhibitory effect of tumor cell-derived lactic acid
45 on human T cells. *Blood*. 2007;109:3812-3819
- 46 206. Bueno V, Binet I, Steger U, et al. The specific monocarboxylate transporter (MCT1)
47 inhibitor, AR-C117977, a novel immunosuppressant, prolongs allograft survival in the mouse.
48 *Transplantation*. 2007;84:1204-1207

- 1 207. Kurita-Ochiai T, Ochiai K, Fukushima K. Butyric acid-induced T-cell apoptosis is
2 mediated by caspase-8 and-9 activation in a Fas-independent manner. *Clin Diagn Lab Immun.*
3 2001;8:325-332
- 4 208. Bailon E, Cueto-Sola M, Utrilla P, et al. Butyrate in vitro immune-modulatory effects
5 might be mediated through a proliferation-related induction of apoptosis. *Immunobiology.*
6 2010;215:863-873
- 7 209. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the
8 differentiation of colonic regulatory T cells. *Nature.* 2013;504:446-+
- 9 210. Klampfer L, Huang J, Sasazuki T, et al. Inhibition of interferon gamma signaling by the
10 short chain fatty acid butyrate. *Mol Cancer Res.* 2003;1:855-862
- 11 211. Stempelj M, Kedinger M, Augenlicht L, et al. Essential role of the JAK/STAT1 signaling
12 pathway in the expression of inducible nitric-oxide synthase in intestinal epithelial cells and its
13 regulation by butyrate. *J Biol Chem.* 2007;282:9797-9804
- 14 212. Boosalis MS, Bandyopadhyay R, Bresnick EH, et al. Short-chain fatty acid derivatives
15 stimulate cell proliferation and induce STAT-5 activation. *Blood.* 2001;97:3259-3267
- 16 213. Jackson SK, DeLoose A, Gilbert KM. Induction of anergy in Th1 cells associated with
17 increased levels of cyclin-dependent kinase inhibitors p21(Cip1) and p27(Kip1). *Journal of*
18 *Immunology.* 2001;166:952-958
- 19 214. Jackson SK, DeLoose A, Gilbert KM. The ability of antigen, but not interleukin-2, to
20 promote n-butyrate-induced T helper 1 cell anergy is associated with increased expression and
21 altered association patterns of cyclin-dependent kinase inhibitors. *Immunology.* 2002;106:486-
22 495
- 23 215. Zhang M, Zhou Q, Dorfman RG, et al. Butyrate inhibits interleukin-17 and generates Tregs
24 to ameliorate colorectal colitis in rats. *BMC Gastroenterol.* 2016;16:84
- 25 216. Schmidt A, Eriksson M, Shang MM, et al. Comparative Analysis of Protocols to Induce
26 Human CD4+Foxp3+ Regulatory T Cells by Combinations of IL-2, TGF-beta, Retinoic Acid,
27 Rapamycin and Butyrate. *PloS one.* 2016;11:e0148474
- 28 217. Mamontov P., Neiman E., Cao T., et al. Effects of short chain fatty acids and GPR43
29 stimulation on human Treg function. . *The Journal of Immunology.* 2015;194:58.14
- 30 218. Asarat M, Apostolopoulos V, Vasiljevic T, et al. Short-Chain Fatty Acids Regulate
31 Cytokines and Th17/Treg Cells in Human Peripheral Blood Mononuclear Cells in vitro.
32 *Immunol Invest.* 2016:1-18
- 33 219. Yang BH, Hagemann S, Mamareli P, et al. Foxp3(+) T cells expressing RORgammat
34 represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during
35 intestinal inflammation. *Mucosal immunology.* 2016;9:444-457
- 36 220. Chaudhry A, Rudra D, Treuting P, et al. CD4+ regulatory T cells control TH17 responses
37 in a Stat3-dependent manner. *Science.* 2009;326:986-991
- 38 221. Marson A, Kretschmer K, Frampton GM, et al. Foxp3 occupancy and regulation of key
39 target genes during T-cell stimulation. *Nature.* 2007;445:931-935
- 40 222. Wang LQ, Tao R, Hancock WW. Using histone deacetylase inhibitors to enhance
41 Foxp3(+) regulatory T-cell function and induce allograft tolerance. *Immunol Cell Biol.*
42 2009;87:195-202
- 43 223. Beier UH, Wang LQ, Hancock WW. Combination of isoform-selective histone/protein
44 deacetylase inhibitors improves Foxp3+T-regulatory cell function. *Cell Cycle.* 2012;11:3351-
45 3352
- 46 224. Kalekar LA, Schmiel SE, Nandiwada SL, et al. CD4 T cell anergy prevents autoimmunity
47 and generates regulatory T cell precursors. *Nat Immunol.* 2016
- 48 225. Shi LZ, Wang R, Huang G, et al. HIF1alpha-dependent glycolytic pathway orchestrates a
49 metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med.*
50 2011;208:1367-1376

- 1 226. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of
2 immunity during autoimmune and infectious diseases. *Nature*. 2014;507:366-+
- 3 227. Kim MS, Kim TS. IgA+ plasma cells in murine intestinal lamina propria as a positive
4 regulator of Treg differentiation. *Journal of leukocyte biology*. 2014;95:461-469
- 5 228. Shi YH, Xu LZ, Peng KS, et al. Specific immunotherapy in combination with *Clostridium*
6 *butyricum* inhibits allergic inflammation in the mouse intestine. *Sci Rep*. 2015;5
- 7 229. Liao HY, Tao L, Zhao J, et al. *Clostridium butyricum* in combination with specific
8 immunotherapy converts antigen-specific B cells to regulatory B cells in asthmatic patients. *Sci*
9 *Rep*. 2016;6:20481
- 10 230. Sindhava VJ, Bondada S. Multiple regulatory mechanisms control B-1 B cell activation.
11 *Front Immunol*. 2012;3:372
- 12 231. Barnes MJ, Powrie F. Regulatory T cells reinforce intestinal homeostasis. *Immunity*.
13 2009;31:401-411
- 14 232. Zhang ZY, Zhang Z, Schluesener HJ. MS-275, an histone deacetylase inhibitor, reduces
15 the inflammatory reaction in rat experimental autoimmune neuritis. *Neuroscience*.
16 2010;169:370-377
- 17 233. Fernando MR, Saxena A, Reyes JL, et al. Butyrate enhances antibacterial effects while
18 suppressing other features of alternative activation in IL-4-induced macrophages. *Am J Physiol*
19 *Gastrointest Liver Physiol*. 2016;ajpgi 00440 02015
- 20 234. Ji J, Shu D, Zheng M, et al. Microbial metabolite butyrate facilitates M2 macrophage
21 polarization and function. *Sci Rep*. 2016;6:24838
- 22 235. Tiemessen MM, Jagger AL, Evans HG, et al. CD4(+)CD25(+)Foxp3(+) regulatory T cells
23 induce alternative activation of human monocytes/macrophages. *P Natl Acad Sci USA*.
24 2007;104:19446-19451
- 25 236. Han S, Lu J, Zhang Y, et al. HDAC inhibitors TSA and sodium butyrate enhanced the
26 human IL-5 expression by altering histone acetylation status at its promoter region. *Immunol*
27 *Lett*. 2007;108:143-150
- 28 237. Raker VK, Domogalla MP, Steinbrink K. Tolerogenic Dendritic Cells for Regulatory T
29 Cell Induction in Man. *Front Immunol*. 2015;6
- 30 238. Goverse G, Molenaar R, Macia L, et al. Diet-Derived Short Chain Fatty Acids Stimulate
31 Intestinal Epithelial Cells To Induce Mucosal Tolerogenic Dendritic Cells. *Journal of*
32 *Immunology*. 2017;198:2172-2181
- 33 239. Schilderink R, Verseijden C, Seppen J, et al. The SCFA butyrate stimulates the epithelial
34 production of retinoic acid via inhibition of epithelial HDAC. *Am J Physiol Gastrointest Liver*
35 *Physiol*. 2016;ajpgi 00411 02015
- 36 240. Cortez VS, Cervantes-Barragan L, Robinette ML, et al. Transforming Growth Factor-beta
37 Signaling Guides the Differentiation of Innate Lymphoid Cells in Salivary Glands. *Immunity*.
38 2016
- 39 241. Viant C, Rankin LC, Girard-Madoux MJ, et al. Transforming growth factor-beta and
40 Notch ligands act as opposing environmental cues in regulating the plasticity of type 3 innate
41 lymphoid cells. *Sci Signal*. 2016;9:ra46
- 42 242. Jang YS, Kim SH, Lee HY. Metabolites from commensal microbes are closely associated
43 with maintaining mucosal homeostasis through modulation of innate lymphoid cells in Peyer's
44 patch. *Journal of Immunology*. 2014;192:133.125
- 45 243. Wohlfert EA, Grainger JR, Bouladoux N, et al. GATA3 controls Foxp3(+) regulatory T
46 cell fate during inflammation in mice. *J Clin Invest*. 2011;121:4503-4515
- 47 244. Mantel PY, Kuipers H, Boyman O, et al. GATA3-driven Th2 responses inhibit TGF-beta1-
48 induced FOXP3 expression and the formation of regulatory T cells. *Plos Biol*. 2007;5:e329

- 1 245. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-
2 inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease
3 patients. *P Natl Acad Sci USA*. 2008;105:16731-16736
- 4 246. De Cruz P, Prideaux L, Wagner J, et al. Characterization of the gastrointestinal microbiota
5 in health and inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:372-390
- 6 247. Lepage P, Hasler R, Spehlmann ME, et al. Twin Study Indicates Loss of Interaction
7 Between Microbiota and Mucosa of Patients With Ulcerative Colitis. *Gastroenterology*.
8 2011;141:227-236
- 9 248. Kamada N, Seo SU, Chen GY, et al. Role of the gut microbiota in immunity and
10 inflammatory disease. *Nat Rev Immunol*. 2013;13:321-335
- 11 249. Clemente JC, Ursell LK, Parfrey LW, et al. The Impact of the Gut Microbiota on Human
12 Health: An Integrative View. *Cell*. 2012;148:1258-1270
- 13 250. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or
14 down? *World J Gastroenterol*. 2006;12:6102-6108
- 15 251. Abraham C, Cho JH. Mechanisms of Disease Inflammatory Bowel Disease. *New Engl J*
16 *Med*. 2009;361:2066-2078
- 17 252. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev*
18 *Immunol*. 2008;8:458-466
- 19 253. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology*.
20 1998;115:182-205
- 21 254. Fujino S, Andoh A, Bamba S, et al. Increased expression of interleukin 17 in inflammatory
22 bowel disease. *Gut*. 2003;52:65-70
- 23 255. Egan CE, Maurer KJ, Cohen SB, et al. Synergy between intraepithelial lymphocytes and
24 lamina propria T cells drives intestinal inflammation during infection. *Mucosal immunology*.
25 2011;4:658-670
- 26 256. Laffont S, Siddiqui KRR, Powrie F. Intestinal inflammation abrogates the tolerogenic
27 properties of MLN CD103(+) dendritic cells. *Eur J Immunol*. 2010;40:1877-1883
- 28 257. Rimoldi M, Chieppa M, Larghi P, et al. Monocyte-derived dendritic cells activated by
29 bacteria or by bacteria-stimulated epithelial cells are functionally different. *Blood*.
30 2005;106:2818-2826
- 31 258. Sayed BA, Brown MA. Mast cells as modulators of T-cell responses. *Immunol Rev*.
32 2007;217:53-64
- 33 259. Powell N, Walker AW, Stolarczyk E, et al. The Transcription Factor T-bet Regulates
34 Intestinal Inflammation Mediated by Interleukin-7 Receptor(+) Innate Lymphoid Cells.
35 *Immunity*. 2012;37:674-684
- 36 260. Ermann J, Staton T, Glickman JN, et al. Nod/Ripk2 signaling in dendritic cells activates
37 IL-17A-secreting innate lymphoid cells and drives colitis in T-bet(-/-).Rag2(-/-) (TRUC) mice.
38 *P Natl Acad Sci USA*. 2014;111:E2559-E2566
- 39 261. Buonocore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-
40 dependent innate intestinal pathology. *Nature*. 2010;464:1371-1375
- 41 262. Bernink JH, Peters CP, Munneke M, et al. Human type 1 innate lymphoid cells accumulate
42 in inflamed mucosal tissues. *Nat Immunol*. 2013;14:221-229
- 43 263. Geremia A, Arancibia-Carcamo CV, Fleming MPP, et al. IL-23-responsive innate
44 lymphoid cells are increased in inflammatory bowel disease. *J Exp Med*. 2011;208:1127-1133
- 45 264. Burgio VL, Fais S, Boirivant M, et al. Peripheral monocyte and naive T-cell recruitment
46 and activation in Crohn's disease. *Gastroenterology*. 1995;109:1029-1038
- 47 265. Choy MY, Walker-Smith JA, Williams CB, et al. Differential expression of CD25
48 (interleukin-2 receptor) on lamina propria T cells and macrophages in the intestinal lesions in
49 Crohn's disease and ulcerative colitis. *Gut*. 1990;31:1365-1370

- 1 266. Volk BA, Niessner M, Amann B, et al. Expression of gamma delta T lymphocytes derived
2 from human intestinal biopsies. *Immunol Res.* 1991;10:310-312
- 3 267. Fukushima K, Masuda T, Ohtani H, et al. Immunohistochemical characterization,
4 distribution, and ultrastructure of lymphocytes bearing T-cell receptor gamma/delta in
5 inflammatory bowel disease. *Gastroenterology.* 1991;101:670-678
- 6 268. McVay LD, Bachwich D, Lichtenstein G, et al. Changes in human mucosal gamma delta
7 T cell repertoire and function are associated with the disease process in inflammatory bowel
8 disease. *Gastroenterology.* 1997;112:A1038-A1038
- 9 269. Nishimura M, Kuboi Y, Muramoto K, et al. Chemokines as Novel Therapeutic Targets for
10 Inflammatory Bowel Disease. *Ann Ny Acad Sci.* 2009;1173:350-356
- 11 270. Papadakis KA, Prehn J, Moreno ST, et al. CCR9-positive lymphocytes and thymus-
12 expressed chemokine distinguish small bowel from colonic Crohn's disease. *Gastroenterology.*
13 2001;121:246-254
- 14 271. Fonseca DM, Hand TW, Han SJ, et al. Microbiota-Dependent Sequelae of Acute Infection
15 Compromise Tissue-Specific Immunity. *Cell.* 2015;163:354-366
- 16 272. Hand TW, Dos Santos LM, Bouladoux N, et al. Acute Gastrointestinal Infection Induces
17 Long-Lived Microbiota-Specific T Cell Responses. *Science.* 2012;337:1553-1556
- 18 273. Kamdar K, Khakpour S, Chen J, et al. Genetic and Metabolic Signals during Acute Enteric
19 Bacterial Infection Alter the Microbiota and Drive Progression to Chronic Inflammatory
20 Disease. *Cell Host Microbe.* 2016;19:21-31
- 21 274. Frank DN, Robertson CE, Hamm CM, et al. Disease Phenotype and Genotype Are
22 Associated with Shifts in Intestinal-associated Microbiota in Inflammatory Bowel Diseases.
23 *Inflamm Bowel Dis.* 2011;17:179-184
- 24 275. Frank DN, Amand ALS, Feldman RA, et al. Molecular-phylogenetic characterization of
25 microbial community imbalances in human inflammatory bowel diseases. *P Natl Acad Sci*
26 *USA.* 2007;104:13780-13785
- 27 276. Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa
28 associated bacterial microflora in patients with active inflammatory bowel disease. *Gut.*
29 2004;53:685-693
- 30 277. Morgan XC, Tickle TL, Sokol H, et al. Dysfunction of the intestinal microbiome in
31 inflammatory bowel disease and treatment. *Genome Biol.* 2012;13
- 32 278. Pozuelo M, Panda S, Santiago A, et al. Reduction of butyrate- and methane-producing
33 microorganisms in patients with Irritable Bowel Syndrome. *Sci Rep.* 2015;5:12693
- 34 279. Varela E, Manichanh C, Gallart M, et al. Colonisation by *Faecalibacterium prausnitzii* and
35 maintenance of clinical remission in patients with ulcerative colitis. *Aliment Pharm Ther.*
36 2013;38:151-161
- 37 280. Machiels K, Joossens M, Sabino J, et al. A decrease of the butyrate-producing species
38 *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with
39 ulcerative colitis. *Gut.* 2014;63:1275-1283
- 40 281. Wang W, Chen LP, Zhou R, et al. Increased Proportions of *Bifidobacterium* and the
41 *Lactobacillus* Group and Loss of Butyrate-Producing Bacteria in Inflammatory Bowel Disease.
42 *J Clin Microbiol.* 2014;52:398-406
- 43 282. Gevers D, Kugathasan S, Denson LA, et al. The Treatment-Naive Microbiome in New-
44 Onset Crohn's Disease. *Cell Host Microbe.* 2014;15:382-392
- 45 283. Sokol H, Seksik P, Furet JP, et al. Low Counts of *Faecalibacterium prausnitzii* in Colitis
46 Microbiota. *Inflamm Bowel Dis.* 2009;15:1183-1189
- 47 284. Treem WR, Ahsan N, Shoup M, et al. Fecal Short-Chain Fatty-Acids in Children with
48 Inflammatory Bowel-Disease. *J Pediatr Gastr Nutr.* 1994;18:159-164
- 49 285. Marchesi JR, Holmes E, Khan F, et al. Rapid and noninvasive metabonomic
50 characterization of inflammatory bowel disease. *J Proteome Res.* 2007;6:546-551

- 1 286. Eeckhaut V, Machiels K, Perrier C, et al. Butyricicoccus pullicaecorum in inflammatory
2 bowel disease. *Gut*. 2013;62:1745-1752
- 3 287. Turrone F, Milani C, Duranti S, et al. Deciphering bifidobacterial-mediated metabolic
4 interactions and their impact on gut microbiota by a multi-omics approach. *Isme J*. 2016
- 5 288. Harig JM, Soergel KH, Komorowski RA, et al. Treatment of diversion colitis with short-
6 chain-fatty acid irrigation. *N Engl J Med*. 1989;320:23-28
- 7 289. Scheppach W, Bartram HP, Richter F, et al. Treatment of distal ulcerative colitis with
8 short-chain fatty acid enemas - A placebo-controlled trial. *Digest Dis Sci*. 1996;41:2254-2259
- 9 290. Scheppach W, Sommer H, Kirchner T, et al. Effect of Butyrate Enemas on the Colonic
10 Mucosa in Distal Ulcerative-Colitis. *Gastroenterology*. 1992;103:51-56
- 11 291. Breuer RI, Buto SK, Christ ML, et al. Rectal Irrigation with Short-Chain Fatty-Acids for
12 Distal Ulcerative-Colitis - Preliminary-Report. *Digest Dis Sci*. 1991;36:185-187
- 13 292. Thibault R, De Coppet P, Daly K, et al. Down-regulation of the monocarboxylate
14 transporter 1 is involved in butyrate deficiency during intestinal inflammation.
15 *Gastroenterology*. 2007;133:1916-1927
- 16 293. Borthakur A, Anbazhagan AN, Kumar A, et al. The probiotic *Lactobacillus plantarum*
17 counteracts TNF- α -induced downregulation of SMCT1 expression and function. *Am J*
18 *Physiol Gastrointest Liver Physiol*. 2010;299:G928-934
- 19 294. Mathewson ND, Jenq R, Mathew AV, et al. Gut microbiome-derived metabolites modulate
20 intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol*. 2016;
21 17:505-13.
- 22 295. Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory
23 CD4⁺CD25^(high) T cells in inflammatory bowel disease. *Gastroenterology*. 2005;128:1868-
24 1878
- 25 296. Holmen N, Lundgren A, Lundin S, et al. Functional CD4⁺CD25^{high} regulatory T cells
26 are enriched in the colonic mucosa of patients with active ulcerative colitis and increase with
27 disease activity. *Inflamm Bowel Dis*. 2006;12:447-456
- 28 297. Saruta M, Yu QT, Fleshner PR, et al. Characterization of FOXP3⁽⁺⁾CD4⁽⁺⁾ regulatory T
29 cells in Crohn's disease. *Clin Immunol*. 2007;125:281-290
- 30 298. Rubtsov YP, Niec RE, Josefowicz S, et al. Stability of the Regulatory T Cell Lineage in
31 Vivo. *Science*. 2010;329:1667-1671
- 32 299. Zhou L, Lopes JE, Chong MM, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell
33 differentiation by antagonizing ROR γ function. *Nature*. 2008;453:236-240
- 34 300. Koenecke C, Czeloth N, Bubke A, et al. Alloantigen-specific de novo-induced Foxp3⁺
35 Treg revert in vivo and do not protect from experimental GVHD. *Eur J Immunol*.
36 2009;39:3091-3096
- 37 301. Hoechst B, Gamrekashvili J, Manns MP, et al. Plasticity of human Th17 cells and iTregs
38 is orchestrated by different subsets of myeloid cells. *Blood*. 2011;117:6532-6541
- 39 302. Xiao Y, Li B, Zhou ZC, et al. Histone acetyltransferase mediated regulation of FOXP3
40 acetylation and Treg function. *Curr Opin Immunol*. 2010;22:583-591
- 41 303. Marino E, Richards JL, McLeod KH, et al. Gut microbial metabolites limit the frequency
42 of autoimmune T cells and protect against type 1 diabetes. *Nat Immunol*. 2017
- 43 304. Pamer EG. Fecal microbiota transplantation: effectiveness, complexities, and lingering
44 concerns. *Mucosal immunology*. 2014;7:210-214
- 45 305. Vermeire S, Joossens M, Verbeke K, et al. Donor Species Richness Determines Faecal
46 Microbiota Transplantation Success in Inflammatory Bowel Disease. *J Crohns Colitis*.
47 2016;10:387-394
- 48 306. Gibson PR. The intracellular target of butyrate's actions: HDAC or HDON'T? *Gut*.
49 2000;46:447-448

- 1 307. Heidor R, Ortega JF, de Conti A, et al. Anticarcinogenic actions of tributyrin, a butyric
2 acid prodrug. *Current drug targets*. 2012;13:1720-1729
- 3 308. Reid T, Valone F, Lipera W, et al. Phase II trial of the histone deacetylase inhibitor
4 pivaloyloxymethyl butyrate (Pivanex, AN-9) in advanced non-small cell lung cancer. *Lung*
5 *Cancer*. 2004;45:381-386
- 6 309. Kang SN, Lee E, Lee MK, et al. Preparation and evaluation of tributyrin emulsion as a
7 potent anti-cancer agent against melanoma. *Drug Deliv*. 2011;18:143-149
- 8
- 9

1 **FIGURE LEGENDS**

2

3 **Figure 1** - The crosstalk between microbiota and the immune system: butyrate (BT) take center
4 stage. BT function directly on intestinal epithelial cells, where they serve as an energy source
5 and have a anti-inflammatory effect. In addition, act directly on immune cells, BT inhibit T
6 cells recruitment, proliferation and induced apoptosis, inhibits T cell activation and increases
7 the number and activity of immunosuppressive Tregs. BT inhibits activation and activity of
8 neutrophils, macrophages, dendritic cells and T cells, thereby eliminating the source of
9 inflammation in the gut tissue. These findings elucidate pathways in which the BT mediates
10 down-regulation of proinflammatory effectors and suggest that microbiota dysbiosis and
11 reduced gut BT concentration may be involved in the pathogenesis of IBD.

12

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

Figure 1

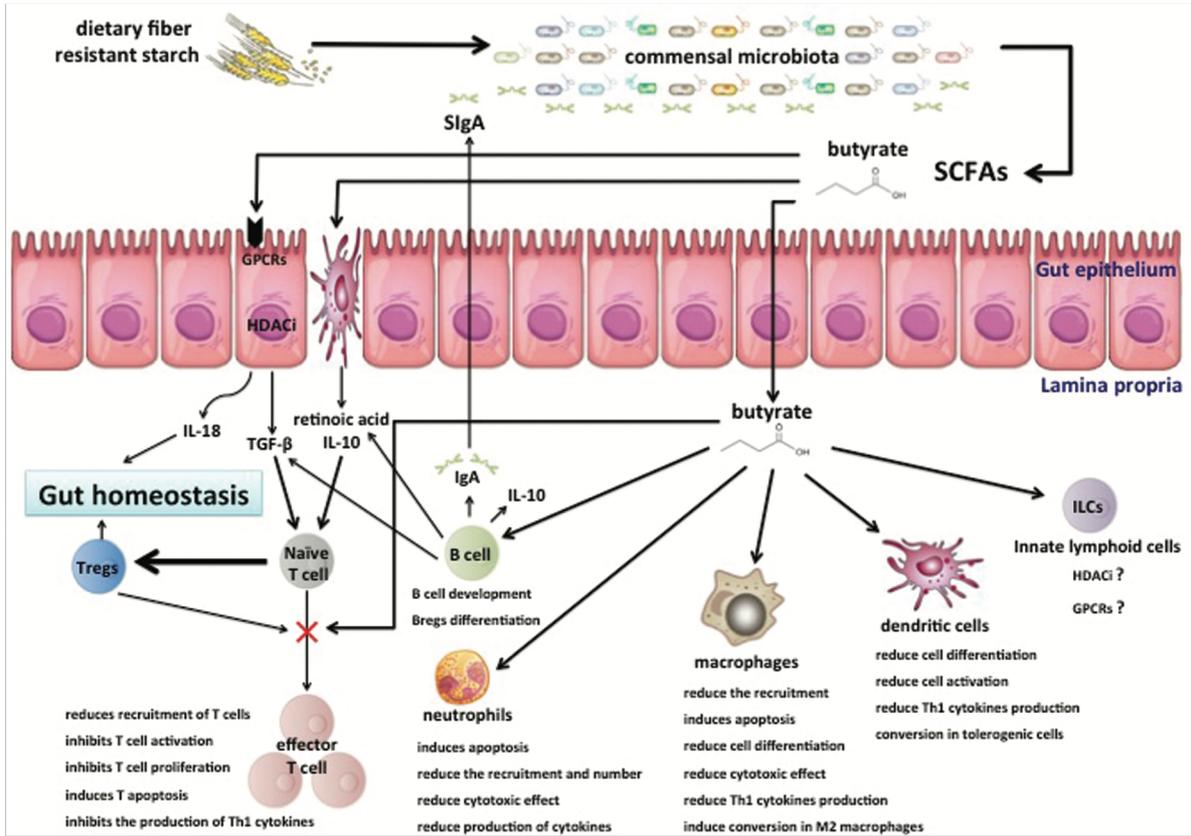


Figure 2

