Manipulation of epithelial integrity and mucosal immunity by host and microbiota-derived metabolites

Hisako Kayama1,2,3 and Kiyoshi Takeda1,2,4

1 Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, Osaka, Japan
2 WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan
3 Institute for Advanced Co-Creation Studies, Osaka University, Osaka, Japan
4 Integrated Frontier Research for Medical Science Division, Institute for Open and Transdisciplinary Research Initiatives, Osaka University, Osaka, Japan

The human intestinal tract contains a large number of microbes, their metabolites, and potentially harmful food antigens. The intestinal epithelium separates the mucosa where immune cells are located from luminal microbes by expressing various factors that assemble into physical and chemical barriers. In addition to epithelial cells, immune cells are essential for enforcing mucosal barriers through production of inflammatory and anti-inflammatory mediators. Intestinal microbiota, represented by gut ecological communities of living microorganisms, influences maturation and homeostasis of host immune system and contributes to the maintenance of the epithelial integrity with small molecules derived from their metabolism, termed metabolites. In turn, immune cells receive signals from microbiota, and may play key role in maintenance of a healthy bacterial composition and reinforcement of epithelial barrier functions, leading to the establishment of a host-bacterial mutualism. Alterations in the microbiota community and metabolome profiles are observed in patients with various disorders including inflammatory bowel disease. In this review, we will discuss physiological functions of the microbiota and its metabolites in regulating host immune system and reinforcing epithelial barrier functions. Further understanding of these processes will aid in identification of novel therapeutic targets and subsequent development of therapeutic interventions in a range of chronic inflammatory diseases.

Keywords: Bile acid · Gut homeostasis · Microbiota · Short-chain fatty acid · Tryptophan

Introduction

The mammalian intestinal tract harbors multiple microorganisms, including bacteria, fungi, viruses, and archaea, as commensals in the lumen. A single-layered epithelium, composed of diverse cell types, segregates the mucosal immune system from the lumen, in which microbes and potentially harmful food antigens are present by producing several factors including antimicrobial peptides and mucins. In addition to epithelial cells, immune cells, such as IFN-γ-producing Th1 cells, IL-17-producing Th17 cells, Foxp3+ Treg cells, B cells, IgA-producing plasma cells, innate lymphoid cells (ILC), and myeloid cells, are essential for construction of the mucosal barriers. Although inflammatory responses by Th1 and Th17 cells in the gut are mandatory for host defense against invading pathogens [1], aberrant activation of effector cells can cause tissue destruction. Therefore, a balance between inflammatory responses and immunological tolerance against the microorganisms and dietary antigens is elaborately regulated in the intestine [2, 3].
Inflammatory bowel diseases (IBD), namely Crohn’s disease (CD), and ulcerative colitis (UC), are chronic disorders of the gastrointestinal tract with relapsing-remitting course. Previous studies have demonstrated that the corresponding rate of CD in monozygotic twins (50-75%) is higher than that of UC (6-19%) [4]. The significant phenotypic discordance between CD and UC in the twins might allude to the presence of factors associated with onset and/or progression of IBD other than genetic susceptibility. Accumulating evidence has indicated that IBD is a polygenic disease and dysregulation of immune responses to the microbes in genetically susceptible individuals is implicated in the pathogenesis of intestinal inflammation [5, 6].

A previous study has characterized 3.3 million open reading frames that are predicted to be the protein-coding regions in human gut microbiome [7], which is more than 150-fold higher than the number of protein-coding genes in human genome. Microbiota is comprised of $10^{13}$ bacteria, which belong to 500-1000 species, and 98% of the human gut microbiota are occupied by the four microbial phyla including Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria [8]. The host intestine provides ecological niches, including anaerobic and nutrient/substrate-rich environments, for microbial colonization, and the microbiota confers beneficial effects on the host, such as nutrient production, biosynthesis of vitamins, production of steroid hormones and neurotransmitters, energy metabolism, xenobiotic metabolism, and preclusion of invasion of pathogenic bacteria. In germ-free mice, the metabolome profiles in the gut, liver, urine, and plasma are drastically changed [9, 10]. For example, higher levels of creatine in the urine, cholines, andinosines, and myo-scyllino-insitol in the kidney, alanine, and tauro-conjugated bile acids (BAs) in the gut were shown in the metabolite profiles of germ-free mice. Emerging evidence demonstrates that the microbially derived and transformed metabolites play important roles in the host physiology through reinforcement of epithelial barrier functions [11] and regulation of immune cell development and homeostasis [2]. Perturbations of microbiota composition, termed dysbiosis, are observed in a variety of diseases including IBD, diabetes, nonalcoholic fatty liver disease, MS, and rheumatoid arthritis [2, 12]. Impaired mucosal barriers due to dysbiosis are accompanied with alterations in metabolome profiles, allowing translocation of luminal microbes and their components into circulation, causing initiation of weak systemic inflammation. In this review, we highlight recent advances in our understanding of the impact of the microbially derived and transformed metabolites, especially short-chain fatty acids (SCFAs), BAs, and amino acid tryptophan catabolites, on murine gut homeostasis through the regulation of host immunity and maintenance of epithelial integrity.

**Regulation of intestinal epithelial barrier function by intestinal metabolites**

Intestinal epithelium is composed of Lgr5+ stem cells, Paneth cells, enterocytes, goblet cells, M cells, tuft cells, and enteroendocrine cells. To separate the microbiota and pathogens existing in the lumen from the epithelia, a mucus layer organized by goblet cell-secreted mucin 2 (MUC2) functions as a physical barrier in the colon, whereas chemical barrier molecules, including regenerating islet-derived protein 3 (Reg3) family proteins and antimicrobial peptides, such as defensins and cathelicidins, are produced by Paneth cells and enterocytes in the small intestine [11, 13]. Signals from the microbiota and underlying immune cells are required for reinforcement of epithelial barrier functions. Among the metabolites synthesized by the host as well as those produced or transformed by the microbiota, SCFAs, BAs, and tryptophan catabolites have been identified as the major signal molecules that modulate intestinal epithelial integrity (Fig. 1A-C).

**Role of SCFAs in intestinal barrier function**

SCFAs are organic fatty acids that are generated by fermentation of dietary fibers by intestinal commensal bacteria. The major SCFAs, such as acetate synthesized from pyruvate, propionate derived from succinate or lactate, and butyrate generated from acetate or lactate, modulate the epithelial barrier functions through several mechanisms (Fig. 1A). SCFAs interact with cell surface G protein-coupled receptors (GPRs) or are taken up via the transporter SLC5A8 in colonic epithelial cells [14]. In intestinal epithelium, SCFAs regulates proliferation of stem cells, expression of anti-microbial molecules and tight junction molecules, and production of cytokines and chemokines [15]. Recognition of SCFAs by GPRs is essential for the epithelial integrity. The activation of GPR41 or GPR43 by SCFAs elicits the production of cytokines, including TNF-α and IL-6, and chemokines, such as CXCL1 and CXCL2, by colonic epithelial cells, which induce protective immune responses against pathogens through promoting recruitment of neutrophils and development of Th17 cells [16]. GPR109a and GPR43 promotes butyrate/acetate-mediated epithelial cell IL-18 production through continuous Ca2+ mobilization or K+ efflux-mediated hyperpolarization, thereby suppressing dextran sodium sulfate (DSS)-induced colitis [17]. In addition, IL-18 production by colonic epithelial cells through GPR109a [18], NLRP6 inflammasome [19], and activation of caspase 1 [20] is thought to prevent colonic inflammation and carcinogenesis. NLRP1 inflammasome is involved in processing IL-18 in nonhematopoietic cells, which contributes to restriction of colonization of the butyrate-producing Clostridiales and aggravates DSS-induced colitis by accelerating IFN-γ production by Th1 cells [21]. IL-18, together with IL-12, IL-3, or IL-2, activates T cells, mast cells, or NKT cells, respectively [22]. These findings suggest that pleiotropic effects of IL-18 in the intestine, such as proinflammatory and anti-inflammatory properties, may depend on cytokine milieu, cell types of IL-18 producer (e.g. nonhematopoietic cells including epithelial cells and macrophages) and IL-18 receptor-expressing responder (e.g. epithelial cells, Th1 cells, mast cells, and NK cells), cellular processes involved in secretion of IL-18 (e.g. inflammasome types), or microbiota community. Butyrate facilitates expression of antimicrobial molecules, including β-defensin and Reg3y, through GPR43-mediated activation of...
transcription factor STAT3 and mTOR in a homeostatic condition [23], while it promotes production of cathelicidin LL-37 in the presence of MUC2 in an inflammatory condition [24]. In addition, butyrate evokes production of MUC2 [25, 26] and expression of tight-junction proteins, including zonula occludens-1 (ZO-1) and claudin [25], by inhibiting histone deacetylase (HDAC)1, 4, 7, 9, or 10 in intestinal epithelial cells [26] in homeostatic condition. Differentiated epithelial cells consume butyrate in the colon under steady state, because butyrate inhibits proliferation of epithelial stem and progenitor cells through activation of transcription factor Foxo3 [27].

Lactate and succinate are precursors of SCFAs. In addition, these metabolites are generated through the microbial respiratory pathway of glycolysis and maintains host epithelial integrity and immune responses against intestinal microorganisms. In the colon of starvation-refed mice, high concentration of lactate produced by Lactobacillus murinus facilitates epithelial cell turnover, leading to high susceptibility to carcinogens [28]. Probiotics (VSL#3)-derived lactate enforces barrier integrity of the small intestinal epithelial cells by inducing Wnt3 expression in stromal cells and Paneth cells via activation of the GPR81 signaling [29]. Furthermore, lactate and pyruvate produced by the microbiota promotes dendrite protrusion of CX3CR1+ phagocytes via GPR31 signaling, which enhances uptake of luminal pathogens such as Salmonella [30]. Succinate produced by intestinal bacteria, helminth, and protozoa acts on tuft cells of the small intestine, which triggers production of IL-25 and subsequently initiates type 2 immune responses through activation of group 2 ILC and Th2 cells [31, 32].
Effects of host synthesized and microbially transformed BAs on regulation of epithelial barrier function

Primary BAs, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), synthesized from cholesterol in the liver are conjugated to glycine or taurine and subsequently stored in the gallbladder [33]. Primary BAs help lipid absorption in the small intestine and approximately 95% of them are reabsorbed by epithelial cells of the ileum through the apical sodium-dependent transporter (Asbt) and released into the lamina propria via the transporter OSTα/OSTβ and then returned to the liver [34]. Unabsorbed primary BAs are microbially converted into secondary BAs; CA to deoxycholic acid (DCA), CDCA to Ursodeoxycholic acid (UDCA), and lithocholic acid (LCA) [34] (Fig. 1B).

BAs directly influence the microbiota community and provide resistance to expansion of pathogens. CA and DCA exert direct antimicrobial effects on Bifidobacterium breve and Lactobacillus salivarius by disrupting bacterial membrane [35]. DCA, generated by Clostridium scindens through 7α-dihydroxylation of CA, provides resistance against Clostridium difficile colonization, which causes antibiotics-induced diarrhea [36, 37]. In addition, DCA prevents Campylobacter jejuni-induced colitis by suppressing expression of inflammatory mediators in both epithelial and immune cells [11].

Secondary BAs modulate epithelial barrier functions via binding to their receptors (Fig. 1B). During wound repair in the colon, DCA promotes crypt regeneration through suppression of prostaglandin (PG) E2 production by stimulating farnesoid X receptor (FXR) [38]. In contrast to inhibition of crypt regeneration, PGE2 accelerates re-establishment of epithelial barrier [38-40]. PGI2 and PGE2 synergistically restore barrier function in the ileum by the intracellular Ca2+ and cAMP signaling-dependent mechanisms [41]. Oral administration of a combination of UDCA and LCA enhances expression of tight-junction molecules, including ZO-1 and claudin-1 via the FGF 15-FXR pathway and thereby, promotes mucosal wound repair [42]. UDCA drives migration of enterocytes through activation of the EGF receptor (EGFR)/COX-2 pathway, which is associated with protection against epithelial barrier destruction [43]. Compared to primary BAs, secondary BAs have a higher affinity for the Takeda G protein receptor 5 (TGR5) on the surface of intestinal epithelial cells, macrophages (Mφ), and DCs. In endocrine cells, TGR5-mediated recognition of DCA promotes the release of 5-hydroxytryptamine (5-HT) and calcitonin gene-related peptide, both of which activate colonic peristalsis [44].

Impact of microbial tryptophan metabolites on epithelial barrier functions

The essential amino acid tryptophan is catalyzed through the indole, kynurenine, or serotonin-producing pathways [45]. The gut microbiota mediates the transformation of tryptophan into indole and indole derivates, whereas kynurenine and serotonin are generated via host-dependent pathways[12]. In the intestinal ecosystem, indole produced by bacteria with tryptophanase, such as Escherichia coli and lactobacilli, affects the physiology of nonindole-producing bacteria by inhibiting biofilm formation, reducing their virulence, and increasing antibiotic tolerance [46].

Indole and its derivates, including indole-3-lactic acid (ILA), indole acrylic acid (IA), indole-3-propionic acid (IPA), indole acetic acid (IAA), and indole-3-aldehyde (IAld), have emerged as an agonist of pregnane X receptor (PXR) and aryl hydrocarbon receptor (AHR). Indole-mediated activation of PXR leads to upregulation of occludin and claudins that are responsible for strengthening epithelial tight junction [47-49]. The AHR signaling mediates regulation of epithelial integrity [50] (Fig. 1C).

Ahr deficiency in epithelial cells is linked to an excess of stem cell proliferation by perturbation of the Wnt-β-catenin signaling, which is associated with impairment of full differentiation of epithelial cells, thereby, causing high susceptibility to infection with pathogenic bacteria [51]. Furthermore, indole stimulates colonic enteroendocrine L cells to secrete hormone peptide glucagon-like peptide 1 (GLP-1) [52], which induces insulin secretion and suppresses gastric emptying and food intake [53]. Among indole derivates, IPA reduces the barrier permeability by enhancing expression of tight-junction molecules, including ZO-1, E-cadherin, and claudins, via PXR activation in the presence of indole [49]. Accordingly, gnotobiotic mice colonized with the engineered Clostridium sporogenes with a mutation within the fDc gene, which fail to produce IPA, exhibit increased intestinal permeability with reduced serum and luminal levels of IPA [54]. A indole derivrate IA-producing Peptostreptococcus species promotes differentiation of goblet cells and mucus production [55]. A neurotransmitter tryptamine generated by microbial tryptophan metabolism is a ligand for a G-protein-coupled-receptor serotonin receptor 4 (5-HT₄R) on colonic epithelial cells [56]. Tryptamine initiates anion-mediated fluid secretion in colonic epithelial cells by increasing intracellular levels of cAMP, facilitating gastrointestinal transit [56].

In summary, physical and chemical epithelial barriers organized by mucins and antimicrobial peptides/proteins interfere with interactions of luminal microbes with mucosal immune cells to prevent initiation of aberrant immune reactions and consequent dysbiosis, which are implicated in the pathogenesis of intestinal inflammation. Conversely, epithelial homeostasis is profoundly affected by several signal molecules produced and modified by commensal bacteria and secreted by host immune cells. Therefore, the signaling crosstalk between epithelium, microbiota, and host immune system plays important roles in maintaining epithelial integrity, which mediates the gut homeostasis.

Regulation of host immunity by microbial metabolites

Innate immune cells, including Mφ, DCs, intraepithelial lymphocytes (IELs), and ILCs respond to pathogens before activation of
adaptine immune cells including Th1, Th17, and Foxp3+ Treg cells [57, 58]. Appropriate inflammatory responses evoked by innate and adaptive immunity are crucial for protection of the host against harmful microorganisms in the intestine, whereas aberrant activation of these cells is implicated in the pathogenesis of intestinal inflammation associated with IBD. Therefore, several tolerogenic mechanisms underlying inhibition of inadequate inflammatory responses exist in the gut such as induction of Foxp3+ Treg cells [59]. The potential roles of the intestinal metabolites, mainly SCFAs, BAs, tryptophan catabolites, and adenosine triphosphate (ATP) in shaping host immune system are well characterized (Fig. 2A-D).

**SCFA-dependent induction of anti-inflammatory T cells in the gut**

SCFAs maintain intestinal immunological tolerance by inducing anti-inflammatory CD4+ T cells through several mechanisms (Fig. 2A). The SCFAs-GPR43 signaling in Th1 cells elicits production of anti-inflammatory cytokine IL-10, which is a key molecule for prevention of colitis [60], by upregulating the expression of Blimp-1 via activation of STAT3 and mTOR [61]. Aceate drives activation of the mTOR signaling through acetylation of p70 S6 kinase and phosphorylation of the ribosomal protein S6 by a GPR41/GPR43-independent mechanism and thereby, promotes...
differentiation of IL-10-producing CD4+ T cells as well as Th1 and Th17 cells [62]. In addition to IL-10-producing CD4+ T cells, SCFAs modulate differentiation and function of Foxp3+ Treg cells by inhibiting HDAC activity and mitigate intestinal inflammation. HDAC inhibitory property of propionate via the GPR43 contributes to generation of Foxp3+ Treg cells and promotion of suppressive activity of these cells [63]. Butyrate enhances histone H3 acetylation at the promoter of Foxp3, which accelerates differentiation and maturation of Foxp3+ Treg cells [64, 65]. Butyrate produced by commensal Clostridia elevates transcription factor specificity protein 1 (SP1)-induced expression of transforming growth factor 1 (TGFβ1) in human intestinal epithelial cells by exerting its HDAC inhibitory activity in a GPR41/GPR43/GPR109a-independent fashion, leading to expansion of Treg cells in the intestine [66]. In colonic Mφs and DCs, binding of butyrate to GPR109a boosts IL-10 production, which initiates differentiation of Foxp3+ Treg cells and IL-10-producing CD4+ T cells [18]. In addition, HDAC inhibition by butyrate is required for suppression of DC maturation through reduction of expression of transcription factor PU.1 and RelB [67] and retention of Mφ hyperreactivity to the microbiota [68].

**Effect of BAs on regulation of immune responses**

Secondary BAs exert their immunomodulatory properties by binding to host cell TGR5, FXR, and vitamin D receptor (VDR) (Fig. 2B). LCA generated by microbial modification of CDCA is the most potent TGR5 agonist [69]. The LCA-TGR5 signaling suppresses NLRP3 inflammasome-dependent inflammation through inducing PKA-mediated ubiquitination and phosphorylation of NLRP3 [69]. TGR5 confers anti-inflammatory profiles on intestinal Mφ by driving CREB-mediated production of IL-10, thereby alleviating intestinal inflammation [70]. FXR agonist INT-747 suppresses production of proinflammatory cytokines, including TNF-α, IL-17, and IFN-γ, by mononuclear cells isolated from the intestinal mucosa of patients with IBD [71]. In intestinal Mφ, CDCA inhibits production of inflammatory mediators, such as iNOS, IL-1β, IL-6, and TNF-α, by modifying chromatin structure of the NFκB binding sites through enhancing recruitment of the nuclear receptor corepressor protein (NCoR) in a FXR-dependent fashion, which is associated with prevention of colitis [72]. The secondary BA, 3β-hydroxydeoxycholic acid (isoDCA), activates FXR and provides DCs with anti-inflammatory phenotype, such as decreased expression of proinflammatory cytokines, TLRs, and antigen presentation-related molecules, thereby potentiating generation of Foxp3+ Treg cells [73]. Germ-free mice colonized with the engineered Bacteroides sp. without ability to transform DCA to isoDCA have lower number of RORγt+ Foxp3+ Treg cells in the colon compared to those in mice harboring functional Bacteroides sp. [73]. Some of the primary BAs (CA, CDCA, and USCA) and the secondary BAs (LCA and 3-oxo-LCA) act on Foxp3+ Treg cells [74]. Interaction of BAs with VDR maintains a subset of RORγt+ Foxp3+ Treg cells in the colon specifically under homeostatic condition, which prevents large intestinal inflammation [75]. 3-oxo-LCA also represses differentiation of Th17 cells through direct interaction with a master regulator RORγt, which links to suppression of its transcriptional activity [75]. Among LCA isoforms, isoalloLCA accelerates development of Foxp3+ Treg cells in the ileum [75]. In CD4+ T cells receiving signals via TCR, isoalloLCA enhances mitochondrial ROS production that promotes histone H3K27 acetylation at the promoter of Foxp3 in the presence of TGF-β [75]. Th1 and Th17 cells in the ileal lamina propria highly express an ATP-dependent efflux transporter ATP-binding cassette subfamily B member 1 (ABCB1) [76, 77]. ABCB1 is required for mitigation of oxidative stress induced by taurine-/glycine-conjugated BAs in effector CD4+ T cells [77]. In patients with ileal CD, function of ABCB1 in effector T cells is drastically reduced [77], suggesting that ABCB1 is crucial for T-cell adaptation to intestinal environment, in which BAs are present abundantly.

**Roles of microbially derived tryptophan metabolites in host innate immunity**

Indole-producing *E. coli* is abundant in the intestine for a week after birth, and thereafter the gut microbiota are dominated by *Bifidobacterium* species that produce indole lactic acid (ILA) and then food intake allows colonization of tryptophan-metabolizing bacterial species, including *Lactobacillus*, *Bacteroides*, *Clostridium*, *Ruminococcus*, and *Peptostreptococcus*, in the gut of infants [53]. Tryptophan is catalyzed to the AHR ligand 1-kynurenine (Kyn) by a host enzyme indoleamine-2,3-dioxygenase (IDO). Kyn maintains immune tolerance by inhibiting NK cell proliferation, T-cell proliferation, and DC maturation [78]. IDO-producing DCs stimulated with probiotic *Lactobacillus salivarius* Ln33, and *Lactobacillus rhamnosus* Lr32 drive development of CD4+ CD25+ Treg cells through activation of nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and TLR2 signaling pathways [79]. Several studies have demonstrated that microbial tryptophan metabolites educate host innate immune system via activation of AHR [55, 80-88] (Fig. 2C). The maternally acquired AHR ligands generated by the microbial metabolism induce accumulation of group 3 ILCs (ILC3s) and F4/80+ CD11c+ phagocytes in the small intestine of offspring [80]. Maternal SCFAs affect the development of the nervous and metabolic systems through embryonic GPR41/43 and its disruptions cause metabolic syndrome in postnatal life [89]. A study has shown that lack of *Card9*, an AHR susceptibility gene, leads to dysbiosis with reduced tryptophan-catalyzing bacteria and diminished number of colonic IL-22-producing ILC3, which are required for induction of Reg3 family expression in epithelial cells [81]. Severe intestinal inflammation during DSS administration in *Card9*-deficient mice is improved by either inoculation of a tryptophan metabolite IAA-producing *Lactobacillus* strains or treatment with AHR agonist [81]. Fecal concentration of IAA is greatly diminished in IBD patients with an ABD-associated SNP within *CARD9* gene (rs10781499) [81]. *Lactobacillus reuteri* produces indole derivates and induces differentiation of intestinal CD4+ T cells into CD4+ CD8α+ IELs with regulatory function, through downregulating transcription factor
Thpok by an AHR-dependent mechanism [82]. Maintenance of the number of IELs by diet-derived AHR ligands is required for prevention of bacterial burden by upregulating expression of Reg3 family proteins in intestinal epithelial cells, which leads to suppression of overactivation of immune cells [83]. AId derived from L. reuteri mediates AHR-dependent expression of Il12 in the stomach, providing resistance to colonization of Candida albicans [84]. A microbial tryptophan metabolite indole-3-acetate (13A) attenuates production of proinflammatory cytokines, including TNF-α, IL-1β, and MCP-1, by Mφ stimulated with LPS plus fatty acid via the activation of AHR [85]. In human blood mononuclear cells, IA, but not IPA, suppresses LPS-induced production of IL-6 and IL-1β and facilitates expression of NF-E2-related factor 2 (NRF2)-mediated antioxidant molecules [55]. AHR in host cells binds to pseudomonas aeruginosa-derived quorum sensing molecules, such as phenazines, homoserine lactones, which confers protection against P. aeruginosa infection by inducing expression of proinflammatory cytokines and chemokines [86, 87]. In addition to innate immunity, the AHR signaling controls adaptive immunity. AHR activation in CD4+ T cells promotes development of Th17 cells and enhances their production of cytokines, including IL-22, IL-17A, and IL-17F, which are associated with exacerbation of experimental autoimmune encephalomyelitis [88].

Intestinal extracellular ATP-mediated initiation of inflammatory immune responses

In the intestine, extracellular ATP is released by luminal bacteria such as E. coli, Salmonella, Enterococcus gallinarum, and Enterococcus mundtii [90-92]. Extracellular ATP controls intestinal immune responses via P2X receptors and P2Y receptors (Fig. 2D). CD70+ CD11b+ DCs in the colon induce accumulation of Th17 cells by producing IL-23, IL-6, and TGF-β in response to extracellular ATP derived from commensal bacteria through P2X/P2Y receptors [93]. Autocrine ATP signaling through P2X7 receptor in activated mast cells is associated with pathogenesis of intestinal inflammation [94]. CD patients show increased number of P2X7-expressing mast cells in the colon, suggesting that the mechanisms underlying regulation of luminal and mucosal hydrolysis of ATP are responsible for maintenance of the immune homeostasis [94].

Concentration of extracellular ATP is tightly regulated by ATP-hydrolyzing ectoenzymes including ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs) [95, 96] and ectonucleotide triphosphohydrolases (E-NTPDases) [97] in the intestine (Fig. 2D). Among seven E-NPPs, E-NPP3 is expressed in mast cells [96] and epithelial cells [95]. Enpp3-deficient mice suffer from allergic intestinal inflammation with elevated number of mast cells because of lack of ATP clearance [96]. Introduction of P2xr7 deficiency into Enpp3-deficient mast cells inhibits ATP-induced proliferation and production of proinflammatory cytokines [96], indicating that E-NPP3 is required for inhibition of ATP-dependent activation of mast cells in the intestine [96]. In addition, E-NPP3 in epithelial cells are responsible for inhibition of P2X7-mediated apoptosis of plasmacytoid DCs, existing in the small intestine and Peyer patches [95]. E-NTPDases hydrolyze ATP to ADP and ADP to AMP. Among them, E-NTPD1, also known as CD39, modulates innate and adaptive immune responses. CD39-mediated ATP hydrolysis enhances suppressive activity of Foxp3+ Treg cells [98, 99]. In innate immunity, CD39 suppresses IL-18 production by neutrophils [100], neutrophil chemotaxis [101], and P2X7 receptor-induced production of IL-1β by Mφs [102] through hydrolyzing ATP. E-NTPD7 in epithelial cells of the small intestine is involved in repression of Th17 differentiation through hydrolysis of commensal bacteria-derived luminal ATP, which prevents development of experimental autoimmune encephalomyelitis [97].

In summary, finely tuned signals from the gut microbiota, including its metabolites, direct protective host immunity and, in turn, the intestinal immune cells affect the microbial community, diversity, and metabolic processes. In addition, host genes impact the microbial composition and immune responses against commensal bacteria. Dysbiosis and disrupted immune homeostasis potentially leads to development and/or progression of intestinal inflammation. Therefore, accurate communications among microbiota, intestinal metabolites, and host immune system are essential for maintenance of the gut homeostasis.

Conclusions

Recent advances in metaproteomics, metagenomics, and metatranscriptomics have demonstrated that the intestinal microbiome is associated with the host health and diseases. Dysbiosis accompanied with alterations in metabolome profiles in biological samples, including urine, plasma, and intestine, is observed in patients with several disorders such as IBD, diabetes, obesity, rheumatoid arthritis, and Parkinson’s disease. As summarized in this review, metabolites, which are synthesized by the host or catalyzed by microbiota, mainly SCFAs, BAs, indole, and extracellular ATP, control epithelial integrity and direct host immunity. However, the majority of microbiota-supplied metabolites are not characterized and the effects of a wide range of intestinal metabolites on host physiology remains poorly understood. Therefore, further understanding of the complicated and diverse mechanisms underlying maintenance of the gut homeostasis through interaction between the microbiota, intestinal metabolites, and the immune system/epithelia barrier will aid in the identification of targets for new therapeutic approaches for various disorders.

Acknowledgments: We thank Hiromi Matsui and Yui Magota for secretarial assistance and Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. This work was supported by PRIME, Japan Agency for Medical Research and Development (19gm6210016) (to H.K.) and by
Conflict of Interest: The authors declare no commercial or financial conflict of interest.

References

1. Ma, H., Tao, W. and Zhu, S., T lymphocytes in the intestinal mucosa: defense and tolerance. Cell Mol. Immunol. 2019. 16: 216–224.

2. Tilg, H., Zmora, N., Adolph, T. E. and Elainav, E., The intestinal microbiota fuelling metabolic inflammation. Nat. Rev. Immunol. 2020. 20: 40–54.

3. Na, Y. R., Stakenborg, M., Seok, S. H. and Matteoli, G., Macrophages in intestinal inflammation and resolution: a potential therapeutic target in IBD. Nat. Rev. Gastroenterol. Hepatol. 2019. 16: 531–543.

4. Halme, L., Paavola-SakkI, P., Turunen, U., Lappalainen, M., Farkkila, M. and Kontula, K., Family and twin studies in inflammatory bowel disease. World J. Gastroenterol. 2006. 12: 3668–3672.

5. Ni, J., Wu, G. D., Albenberg, L. and Tomov, V. T., Gut microbiota and IBD: causation or correlation? Nat. Rev. Gastroenterol. Hepatol. 2014. 11: 573–584.

6. Ananthakrishnan, A. N., Epidemiology and risk factors for IBD. Nat. Rev. Gastroenterol. Hepatol. 2015. 12: 205–219.

7. Qin, J., Ji, L., Ri, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bernalier, M., Batto, J. M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H. B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, J., Yang, H., Wang, J., Brunak, S., Dore, J., Guerri, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Meta, H. I. T. C., Bork, P., Ehrlich, S. D. and Wang, J., A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010. 464: 69–75.

8. Cho, I. and Blaser, M. J., The human microbiome: at the interface of health and disease. Nat. Rev. Genet. 2012. 13: 260–270.

9. Claus, S. P., Tsang, T. M., Wang, Y., Cloarec, O., Skordi, E., Martin, F. P., Rezzi, S., Ross, A., Kochhar, S., Holmes, E. and Nicholson, J. K., Systematic comparison of effects of the gut microbiome on mouse metabolic phenotypes. Mol. Syst. Biol. 2008. 4: 219.

10. Selwyn, F. F., Csakany, I. L., Zhang, Y. and Kläassen, C. D., Importance of large intestine in regulating bile acids and glucagon-like peptide-1 in germ-free mice. Drug Metab. Dispos. 2015. 43: 1544–1556.

11. Allaire, J. M., Crowley, S. M., Law, H. T., Chang, S. Y., Ko, H. J. and Vallance, B. A., The intestinal epithelium: central coordinator of mucosal immunity. Trends Immunol. 2018. 39: 677–696.

12. Lavelle, A. and Sokol, H., Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. Nat. Rev. Gastroenterol. Hepatol. 2020. 17: 223–237.

13. Barker, N., Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat. Rev. Mol. Cell Biol. 2014. 15: 19–33.

14. Koh, A., De Vadder, F., Kovatcheva-Datchary, P. and Backhed, F., From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 2016. 165: 1332–1345.

15. Rooks, M. G. and Barrett, W. S., Gut microbiota, metabolites and host immunity. Nat. Rev. Immunol. 2016. 16: 341–352.

16. Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M. and Kim, C. H., Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. Gastroenterology 2013. 145: 396–406 e391-310.

17. Macia, L., Tan, J., Vieira, A. T., Leach, K., Stanley, D., Luong, S., Maruya, M., Ian McKenzie, C., Hijikata, A., Wong, C., Bing, L., Thorburn, A. N., Chevalier, N., Ang, C., Marino, E., Robert, R., Offermanns, S., Teixeira, M. M., Moore, R. J., Flavell, R. A., Fagarasan, S. and Mackay, C. R., Metabo-lite-sensing receptors GPR41 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflamma-some. Nat. Commun. 2015. 6: 6734.

18. Singh, N., Gurav, A., Sivaprakasam, S., Brady, E., Padia, R., Shi, H., Thangaraju, M., Prasad, P. D., Manicasamy, S., Munn, D. H., Lee, J. R., Offermanns, S. and Ganapathy, V., Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 2014. 40: 128–139.

19. Elainav, E., Strowig, T., Kau, A. L., Henao-Mejia, J., Thaiss, C. A., Booth, C. J., Peper, D. R., Bertin, J., Eisenbarth, S. C., Gordon, J. I. and Flavell, R. A., NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 2011. 145: 745–757.

20. Dupaul-Chicoine, J., Yeretzian, G., Doiron, K., Bergstrom, K. S., McIntire, C. R., LeBlanc, P. M., Meunier, C., Turbite, C., Gros, P., Beauchemin, N., Vallance, B. A. and Saleh, M., Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. Immunity 2010. 32: 367–378.

21. Tye, H., Yu, C. H., Simms, L. A., de Zoete, M. R., Kim, M. L., Zakrzewski, M., Penington, J. S., Harapas, C. R., Souza-Fonseca-Guimaraes, F., Wockner, L. F., Preau, A., Mielke, L. A., Wilcox, S. A., Ogura, Y., Corr, S. C., Kanojia, K., Kouremonos, K. A., De Souza, D. P., McConville, M. J., Flavell, R. A., Garic, M., Kile, B. T., Papenfuss, A. T., Putockzi, T. L., Radford-Smith, G. L. I. and Masters, S. L., NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat. Commun. 2018. 9: 3728.

22. Yasuda, K., Nakashiki, K. and Tsutsui, H., Interleukin-18 in health and disease. Int. J. Mol. Sci. 2019. 20: 649.

23. Zhao, Y., Chen, F., Wu, W., Sun, M., Bilotta, A. J., Yao, S., Xiao, Y., Huang, X., Eaves-Pyles, T. D., Golovko, G., Romanov, Y., D’Souza, W., Zhao, Q., Liu, Z. and Cong, Y., GPR43 mediates microbiotic metabolic pathway SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of metro and STAT3. Mucosal Immun. 2018. 11: 752–762.

24. Cobo, E. R., Klisoon-Singh, V., Moreau, F., Holani, R. and Chadee, K., MUC2 mucin and butyrate contribute to the synthesis of the antimicrobial peptide cathelicidin in response to Entamoeba histolytica- and dextran sodium sulphate-induced colitis. Infect. Immun. 2017. 85.

25. Gonzalez, A., Krieg, R., Massey, H. D., Carl, D., Ghosh, S., Gehr, T. W. and Ghosh, S. S., Sodium butyrate amelioreses insulin resistance and renal failure in CKD rats by modulating intestinal permeability and mucin expression. Nephrol. Dial. Transplant. 2019. 34: 783–794.

26. Mathewson, N. D., Jeng, R., Mathew, A. V., Koensigknecht, M., Hanah, A., Toubai, T., Oravecz-Wilson, K., Wu, S. R., Sun, Y., Ross, C., Fujiwara, H., Byun, J., Shono, Y., Lindemans, C., Calafore, M., Schmidt, T. M., Honda, K., Young, V. B., Pennathur, S., van den Brink, M. and Reddy, P., Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. Nat. Immunol. 2016. 17: 505–513.

27. Kako, G. E., Ryu, S. H., Kous, O. I., Collins, P. L., Solnica-Krezel, L., Pearce, E. J., Pearce, E. L., Oltz, E. M. and Stappenbeck, T. S., The colonic
IL-25 regulates an intestinal ILC2-epithelial response circuit. Cell Host Microbe 2018. 24: 833–846 e836.

529 IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 2006.

529 Metabolome: implications for health and disease. Nature 2010. 464: 345–369.

656 IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 2019. 566: 110–114.

50 von Moltke, J., Ji, M., Liang, H. E. and Locksley, R. M., Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 2016. 529: 221–225.

613 Nadjsombati, M. S., McGinty, J. W., Lyons-Cohen, M. R., Jaffe, J. B., DiPeso, L., Schneider, C. M., Miller, C. N., Pollack, J. L., Nagana Gowda, G. A., Fontana, M. F., Erle, D. J., Anderson, M. S., Locksley, R. M., Raferty, D. and von Moltke, J., Detection of succinate by intestinal tuft cells triggers a type 2 innate immune circuit. Immunity 2018. 49: 33–41 e37.

3 Van Treuren, W. and Dodd, D., Microbial contribution to the human metabolome: implications for health and disease. Annu. Rev. Pathol. 2015. 10: 345–369.

24 de Aguiar Vallim, T. Q., Tarling, E. J. and Edwards, P. A., Pleiotropic roles of bile acids in metabolism. Cell Metab. 2013. 17: 657–669.

740 Kurdi, P., Kawanishi, K., Mizutani, K. and Yokota, A., Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. J. Bacteriol. 2006. 188: 1979–1986.

506 Greathouse, K. L., Harris, C. C. and Bultman, S. J., Dysfunctional families: Clostridium scordius and secondary bile acids inhibit the growth of Clostridium difficile. Cell Metab. 2015. 21: 9–10.

375 Buffe, C. G., Bucci, V., Stein, R. R., McKenzie, P. T., Ling, L., Gobourne, A., No, D., Liu, H., Kinnebrew, M., Viale, A., Littmann, E., van den Brink, M. R., Jeng, R. R., Taur, Y., Sander, C., Cross, J. R., Toussaint, N. C., Xavier, J. B. and Pamer, E. G., Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature 2015. 517: 205–208.

38 Jain, U., Lai, C. W., Xiong, S., Goodwin, V. M., Lu, Q., Muegge, B. D., Christophi, G. P., VanDussen, K. L., Cummings, B. P., Young, E., Hambor, J. and Stappenbeck, T. S., Temporal regulation of the bacterial metabolite deoxycholate during colonic repair is critical for crypt regeneration. Cell Host Microbe 2018. 24: 353–363 e355.

682 Miyoshi, H., VanDussen, K. L., Malvin, N. P., Ryu, S. H., Wang, Y., Sonnek, N. M., Lai, C. W. and Stappenbeck, T. S., Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. EMBO J. 2017. 36: 5–24.

50 Kim, H. B., Kim, M., Park, Y. S., Park, I., Kim, T., Yang, S. Y., Cho, C. J., Hwang, D., Jung, J. H., Markowitz, S. D., Hwang, S. W., Yang, S. K., Lim, D. S. and Myung, S. J., Prostaglandin E2 activates YAP and a positive-signaling loop to promote colon regeneration after colitis but also carcinogenesis in mice. Gastroenterology 2017. 152: 616–630.

803 Blisklager, A. T., Roberts, M. C., Rhoads, J. M. and Argenzio, R. A., Prostaglandins E2 and E2 have a synergistic role in rescuing epithelial barrier function in porcine ileum. J. Clin. Invest. 1997. 100: 1928–1933.
epithelial G-protein-coupled receptor to increase colonic secretion. Cell Host Microbe 2018. 23: 775–785 e775.

57 Joeris, T., Muller-Luda, K., Agac, W. H. W. and Mowat, A. M., Diversity and functions of intestinal mononuclear phagocytes. Mucosal Immunol 2017. 10: 845–864.

58 Klose, C. S. and Artis, D., Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. Nat. Immunol. 2016. 17: 765–774.

59 Sakaguchi, S., Miyara, M., Costantino, C. M. and Hafler, D. A., FOXP3+ regulatory T cells in the human immune system. Nat. Rev. Immunol. 2010. 10: 490–500.

60 Friedrich, M., Pohlin, M. and Powrie, F., Cytokine networks in the pathophysiology of inflammatoty bowel disease. Immunity 2019. 50: 992–1006.

61 Sun, M., Wu, W., Chen, L., Yang, W., Huang, X., Ma, C., Chen, F., Xiao, Y., Zhao, Y., Ma, C., Yao, S., Carpio, V. H., Dann, S. M., Zhao, Q., Liu, Z. and Cong, Y., Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. Nat. Commun. 2018. 9: 3555.

62 Park, J., Kim, M., Kang, S. G., Jannasch, A. H., Cooper, B., Patterson, J. and Kim, C. H., Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. Mucosal Immunol 2015. 8: 80–93.

63 Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., Glickman, J. N. and Garrett, W. S., The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013. 341: 569–573.

64 Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakashisi, Y., Uetake, C., Kato, K., Takahashi, M., Fukuda, N. N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J. M., Mopping, D. L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase, K. and Ohno, H., Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 2013. 504: 446–450.

65 Arpaia, N., Campbell, C., Fan, X., Diky, S., van der Veeken, J., de Roos, P., Liu, H., Cross, J. R., Pfeffer, K., Coffer, P. J. and Rudensky, A. Y., Metabolites produced by commensal bacteria promote peripheral regulatory T-cell tolerance. Nature 2013. 504: 451–455.

66 Martin-Gallausiaux, C., Beguet-Crespel, F., Marinelli, L., Jamet, A., Ledue, F., Blottiere, H. M. and Lapaque, N., Butyrate produced by gut commensal bacteria activates TGF-beta1 expression through the transcription factor SP1 in human intestinal epithelial cells. Sci. Rep. 2018. 8: 9742.

67 Singh, N., Thangaraju, M., Prasad, P. D., Martin, P. M., Lambert, N. A., Boettger, T., Offermanns, S. and Ganapathy, V., Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. J. Biol. Chem. 2010. 285: 27601–27608.

68 Chang, P. V., Hao, L., Offermanns, S. and Medzhitov, R., The microbrial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc. Natl. Acad. Sci. USA. 2014. 111: 2247–2252.

69 Guo, C., Xie, S., Chi, Z., Zhang, J., Liu, Y., Zhang, L., Zheng, M., Zhang, X., Xia, D., Ke, Y., Lu, L. and Wang, D., Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity 2016. 45: 802–816.

70 Biaglio, M., Carino, A., Cipriani, S., Francisci, D., Marchiano, S., Scarpelli, P., Sornici, D., Zampella, A. and Fiorucci, S., The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from murine colitis. J. Immunol. 2017. 199: 718–733.
intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 2011. 147: 629–640.

84 Zelante, T., Iannitti, R. G., Cunha, C., De Luca, A., Giovannini, G., Pieraccini, G., Zecchi, R., D’Angelo, C., Massi-Benedetti, C., Fallarino, F., Carvalho, A., Puccetti, P. and Romani, L., Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013. 39: 372–385.

85 Krishnan, S., Ding, Y., Saedi, N., Choi, M., Sridharan, G. V., Sherr, D. H., Yarmush, M. L., Alaniz, R. C., Jayaraman, A. and Lee, K., Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. Cell Rep. 2018. 23: 1099–1111.

86 Moura-Alves, P., Fae, K., Houthuys, E., Dorhoi, A., Kreuchwig, A., Furtet, J., Barison, N., Diehl, A., Munder, A., Constant, P., Skrahina, T., Guhlich-Bornhoff, U., Klemm, M., Koehler, A. B., Bandermann, S., Gooss, C., Mollenkopf, H. J., Hurwitz, R., Brinkmann, V., Fillatreau, S., Daffe, M., Tummler, B., Kolbe, M., Oschkinat, H., Krause, G. and Kaufmann, S. H., AhR sensing of bacterial pigments regulates antibacterial defence. Nature 2014. 512: 387–392.

87 Moura-Alves, P., Puyskens, A., Stinn, A., Klemm, M., Guhlich-Bornhoff, U., Dorhoi, A., Furtet, J., Kreuchwig, A., Protez, J., Lozza, L., Pei, G., Saikali, P., Perdomo, C., Mollenkopf, H. J., Hurwitz, R., Kirschhoefer, F., Brenner-Weiss, G., Weiner, J., 3rd, Oschkinat, H., Kolbe, M., Krause, G. and Kaufmann, S. H. E., Host monitoring of quorum sensing during Pseudomas aeruginosa infection. Science. 2019. 366: eaaw1629.

88 Veldhoen, M., Hirota, K., Westendorf, A. M., Buer, J., Dumoutier, L., Renaud, J. C. and Stockinger, B., The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 2008. 453: 106–109.

89 Kimura, I., Miyamoto, J., Ohue-Kitano, R., Watanabe, K., Yamada, T., Onuki, M., Aoki, R., Isobe, Y., Kashihara, D., Inoue, D., Inaba, A., Taka-mura, Y., Taira, S., Kumaki, S., Watanabe, M., Ito, M., Nakagawa, F., Irie, J., Kakuta, H., Shinohara, M., Iwatsuki, K., Tsujimoto, G., Ohno, H., Arita, M., Itoh, H. and Hase, K., Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. Science. 2020. 367.

90 Hironaka, I., Iwase, T., Sugimoto, S., Okuda, K., Tajima, A., Yanaga, K. and Mizuno, Y., Glucose triggers ATP secretion from bacteria in a growth-phase-dependent manner. Appl. Environ. Microbiol. 2013. 79: 2328–2335.

91 Mempin, R., Tran, H., Chen, C., Gong, H., Kim Ho, K. and Lu, S., Release of extracellular ATP by bacteria during growth. BMC Microbiol. 2013. 13: 301.

92 Iwase, T., Shinji, H., Tajima, A., Sato, F., Tamura, T., Iwasato, T., Yoneda, M. and Mizuno, Y., Isolation and identification of ATP-secreting bacteria from mice and humans. J. Clin. Microbiol. 2010. 48: 1949–1951.

93 Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., Yagita, H., Ishii, N., Evans, R., Honda, K. and Takeda, K., ATP drives lamina propria TH17 cell differentiation. Nature. 2008. 455: 808–812.

94 Kurashima, Y., Amiya, T., Noch, T., Fujisawa, K., Haraguchi, T., Iba, H., Tsutsumi, H., Sato, F., Nakajima, S., Iijima, H., Kubo, M., Kumisawa, J. and Kiyono, H., Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoreceptors. Nat. Commun. 2012. 3: 1034.

95 Furuta, Y., Tsai, S. H., Kinoshita, M., Fujimoto, K., Okumura, R., Unemoto, E., Kurashima, Y., Kiyono, H., Kayama, H. and Takeda, K., E-NTPP controls plasmacytoid dendritic cell numbers in the small intestine. PLoS One 2017. 12: e0172509.

96 Tsai, S. H., Kinoshita, M., Kusut, T., Kayama, H., Okumura, R., Ikeda, K., Shimada, Y., Takeda, A., Yoshikawa, S., Obata-Ninomiya, K., Kurashima, Y., Sato, S., Unemoto, E., Kiyono, H., Karasuyama, H. and Takeda, K., The ectoenzyme E-NTPP negatively regulates ATP-dependent chronic allergic responses by basophils and mast cells. Immunity. 2015. 42: 279–293.

97 Kusu, T., Kayama, H., Kinoshita, M., Jeon, S. G., Ueda, Y., Goto, Y., Okumura, R., Saiga, H., Kurakawa, T., Ikeda, K., Maeda, Y., Nishimura, J., Arima, Y., Atarashi, K., Honda, K., Murakami, M., Kuni-sawa, J., Kiyono, H., Okumura, Y., Yamamoto, M. and Takeda, K., Ecto-nucleoside triphosphate diphosphohydrolase 7 controls TH17 cell responses through regulation of luminal ATP in the small intestine. J. Immunol. 2013. 190: 774–783.

98 Borselli, G., Kleine-Wittefeld, M., Di Mitri, D., Sternjak, A., Diamantini, A., Giometto, R., Hopner, S., Centzonoe, D., Bernardi, G., Dell’Acqua, M. L., Rossini, F. M., Battistini, L., Rotzschke, O. and Falk, K., Expression of ectonucleotidase CD39 by Foxxp3+ T-cells: hydrolysis of extracellular ATP and immune suppression. Blood 2007. 110: 1225–1232.

99 Deaglio, S., Dwyer, K. M., Gao, W., Friedman, D., Usheva, A., Erat, A., Chen, J. F., Enjoji, K., Linden, J., Oukka, M., Kuchroo, V. K., Strom, T. B. and Robson, S. C., Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J. Exp. Med. 2007. 204: 1257–1265.

100 Kukulski, F., Brahmi, F., Ben Yebsri, F., Levesque, S. A. and Sevigny, J., NTPDase1 controls IL-8 production by human neutrophils. J. Immunol. 2011. 187: 644–653.

101 Corrden, R., Chen, Y., Inoue, Y., Beldi, G., Robson, S. C., Insel, P. A. and Junger, W. G., Ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1/CD39) regulates neutrophil chemotaxis by hydrolyzing released ATP to adenosine. J. Biol. Chem. 2008. 283: 28480–28486.

102 Levesque, S. A., Kukulski, F., Enjoji, K., Robson, S. C. and Sevigny, J., NTPDase1 governs P2X7-dependent functions in murine macrophages. Eur. J. Immunol. 2010. 40: 1473–1485.

Abbreviations: CA: cholonic acid CD: Crohn’s disease CDCA: chenodeoxycholic acid DSS: dextran sodium sulfate FXR: farnesoid X receptor GPRs: G protein-coupled receptors HDAC: histone deacetylase 5-HT: 5-hydroxytryptamine IA: indole acetic acid IAId: indole-3-aldehyde IBD: inflammatory bowel diseases ILC: innate lymphoid cells IPA: indole-3-propionic acid SCFAs: short-chain fatty acids TGR5: Takeda G protein receptor 5 UC: ulcerative colitis VDR: vitamin D receptor

Full correspondence: Prof. Kiyoshi Takeda, Laboratory of Immune Regulation, Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. e-mail: ktakeda@ongene.med.osaka-u.ac.jp

Received: 13/4/2020 Revised: 8/5/2020 Accepted: 4/6/2020 Accepted article online: 8/6/2020