

## The gut microbiota — masters of host development and physiology

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**Abstract** | Establishing and maintaining beneficial interactions between the host and its associated microbiota are key requirements for host health. Although the gut microbiota has previously been studied in the context of inflammatory diseases, it has recently become clear that this microbial community has a beneficial role during normal homeostasis, modulating the host's immune system as well as influencing host development and physiology, including organ development and morphogenesis, and host metabolism. The underlying molecular mechanisms of host–microorganism interactions remain largely unknown, but recent studies have begun to identify the key signalling pathways of the cross-species homeostatic regulation between the gut microbiota and its host.

### Microbiota

The sum of all microorganisms (including bacteria, archaea, eukaryotes and viruses) that reside in and/or on a host or a specified part of a host (such as the gastrointestinal tract).

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All higher animals are associated with a diverse microbial community that is composed mainly of bacteria but also includes archaea, viruses, fungi and protozoa. Microorganisms cover essentially all host mucosal surfaces, but most reside within the gastrointestinal tract. Studies had traditionally focused on examining the role of the microbiota during human disease, for example in inflammatory diseases such as colitis. However, in the past decade, the field of microbiota research has exploded, resulting in the publication of a plethora of reports that describe both the individual members of our intestinal microbiota and their wide-ranging impact on host physiology. Thus, the traditional anthropocentric view of the gut microbiota as pathogenic and solely an immunological threat has been substituted with an appreciation of its mainly beneficial influence on human health.

The 'normal' gut microbiota is dominated by anaerobic bacteria, which outnumber aerobic and facultative anaerobic bacteria by 100- to 1,000-fold<sup>1</sup>. In total, the intestinal microbiota consists of approximately 500–1,000 species that, interestingly, belong to only a few of the known bacterial phyla<sup>2,3</sup>. By far the most abundant phyla in the human gut are Firmicutes and Bacteroidetes, but other species present are members of the phyla Proteobacteria, Verrumicrobia, Actinobacteria, Fusobacteria and Cyanobacteria<sup>2,3</sup>. Two gradients of microbial distribution can be found in the gastrointestinal tract. First, microbial density increases both from the proximal to the distal gut (the stomach contains 10<sup>1</sup> microbial cells per gram of content, the duodenum 10<sup>3</sup> cells per gram, the jejunum 10<sup>4</sup> cells per

gram, the ileum 10<sup>7</sup> cells per gram and the colon up to 10<sup>12</sup> cells per gram) and along the tissue–lumen axis (with few bacteria adhering to the tissue or mucus but a large number being present in the lumen)<sup>4</sup>. Second, bacterial diversity increases in the same axes and manner as microbial density<sup>4</sup>. Many bacterial species are present in the lumen, whereas fewer, but well-adapted species, including several proteobacteria and *Akkermansia muciniphila*, adhere and reside within the mucus layer close to the tissue<sup>5,6</sup>. Colonization of the host begins during birth, and the composition of the microbiota changes throughout host development (BOX 1).

In the adult intestine, a total of about 10<sup>14</sup> bacterial cells are present, which is ten times the number of human cells in the body<sup>7</sup>. Their combined genomes (known as the microbiome) contain more than 5 million genes, thus outnumbering the host's genetic potential by two orders of magnitude<sup>2,8</sup>. This large arsenal of gene products provides a diverse range of biochemical and metabolic activities to complement host physiology. In fact, the metabolic capacity of the gut microbiota equals that of the liver, and the intestinal microbiota can therefore be considered as an additional organ<sup>9</sup>. These bacteria are essential for several aspects of host biology. For example, they facilitate the metabolism of otherwise indigestible polysaccharides and produce essential vitamins; they are required for the development and differentiation of the host's intestinal epithelium and immune system; they confer protection against invasion by opportunistic pathogens<sup>10</sup>; and they have a key role in maintaining tissue homeostasis. Recent studies have also revealed that the human microbiota influences

## Box 1 | Colonization of the host

Human babies are colonized during passage through the birth canal by environmental microorganisms (for example, from the mother's vagina or skin) and during breast feeding by microorganisms present in the milk<sup>137</sup>. Owing to the highly oxidative environment in the gastrointestinal tract of the newborn, primary colonizers are facultative anaerobic bacteria such as proteobacteria, which are thought to adjust the environmental conditions by decreasing the oxygen concentration to allow successive colonization by anaerobic microorganisms such as members of the genus *Bacteroides* and members of the phyla Actinobacteria and Firmicutes. During the first year of life, the intestinal microbiota composition is simple and fluctuates widely between individuals and over time. Microbial signatures stabilize and start to resemble the 'adult state' when the infant reaches 1–2 years of age<sup>4</sup>.

Interestingly, conflicting evidence has been published concerning the driving force for microbial transmission. In early studies of twins, the faecal microbial compositions in the mother and her children were similar, indicating a mainly maternal transmission<sup>108,111</sup>. However, in a more recent and extensive study, the same research group found that the faecal microbiota of children was no more similar to that of their mothers than to that of their biological fathers, and genetically unrelated but co-habiting mothers and fathers were significantly more microbially similar to one another than to members of different families<sup>138</sup>. This indicates that, as well as genetics and kinship, environmental factors have a considerable effect on the microbial composition of the infant.

the development and homeostasis of other host tissues, including the bone<sup>11</sup>.

The microbiota also benefits from this mutualistic association, as the mammalian intestine is a nutrient-rich environment that is maintained at a constant temperature. However, it is also a dynamic habitat that undergoes constant and rapid changes in its physiological parameters owing to variations in, for example, host diet, lifestyle, hygiene or use of antibiotics, all of which affect gut microbial composition (FIG. 1). Thus, unlike the host genome, the microbiome can change rapidly as a result of modifications in either the composition of the microbial community or individual microbial genomes, resulting in modified transcriptomic, proteomic and metabolic profiles. Accordingly, the establishment and preservation of beneficial interactions between the host and its associated intestinal microbiota are key requirements for health.

The dynamic fluctuations in the microbiota combined with the vast numbers of bacterial cells and their close proximity to the epithelial tissue represent a massive challenge to host immunity, as microbial growth has to be restricted to ensure a beneficial homeostasis. Furthermore, activation of the host immune system has to be controlled to circumvent the detrimental effects of chronic inflammation, so the interaction of the gut microbiota with the host has to be tightly regulated. In this Review, we discuss recent insights into the impact of the normal microbiota on the development and homeostasis of the immune system and other tissues and organs, as well as on host physiology. We also highlight recent advances in deciphering the underlying molecular mechanisms of host–microorganism interactions.

### Tailoring immune development

Immunology was originally based on the concept of 'self' versus 'non-self' discrimination, with the assumption that, because they are non-self, all microorganisms

are pathogens and thus the cause of infectious diseases. The realization that we live in a microbially dominated world and in fact benefit greatly from our microbiota has led to a paradigm shift in immunology. Thus, the definition of self in the superorganism theory has been extended to incorporate the constituents of both our own body and our microbiota<sup>12</sup>. It is also now widely accepted that the host's mucosal immune system is characterized by tolerance to microorganisms rather than responsiveness<sup>13</sup>. Furthermore, it has even been speculated that the highly sophisticated adaptive immune system of jawed vertebrates evolved to keep control of the mutualistic or beneficial symbiosis with our complex microbial ecosystem<sup>14</sup>.

The intestine, one essential organ in which the mucosal immune system operates, has to accomplish two apparently confounding tasks. First, it needs to facilitate nutrient absorption; thus, the total surface area of the gastrointestinal tract amounts to about 200 m<sup>2</sup> in humans<sup>15</sup>. Second, it needs to be resistant to infection and inhibit microbial translocation across the tissue barrier. Bacterial densities in the gut are the highest known in any habitat to date and reach up to 10<sup>12</sup> cells per gram in the lower intestine<sup>16</sup>. This highly dense microbial community and the host intestinal epithelial cell (IEC) lining are separated by only a few micrometres of mucus in the small intestine and up to several hundred micrometres in the colon, depending on the location<sup>17</sup>. Because of this unique nature of the intestinal tract, its mucosal immune system needs to fulfil several special requirements. It has to be non-responsive to or tolerant towards the huge number of mutualistic microorganisms that reside in the intestinal lumen. At the same time, it is thought that the mucosal immune system has to assure a beneficial microbiota composition by keeping pathogens in check, restricting microbial overgrowth and reacting to penetrating microorganisms that breach the intestinal chemical and physical barriers (such as secreted soluble immunoglobulin A (IgA), antimicrobial peptides (AMPs), the mucus layer and the tightly interconnected IEC lining). In turn, the intestinal microbiota has a key role in directing several aspects in the development and regulation of the host's immune tissues, immune cell populations and immune mediators.

### Mucus layer properties depend on intestinal bacteria.

The intestinal mucus layer covers the epithelial cell lining and functions as a lubricant, facilitating gastrointestinal transport, and as a protective layer against bacterial invasion, owing to its physical properties<sup>18</sup>. The colonic mucus layer is in fact composed of two layers<sup>17</sup>. Both the inner and outer mucus layers are secreted by goblet cells and are mainly made up of gel-forming highly glycosylated proteins termed mucins<sup>18</sup>. Mucin 2 (MUC2) is the main mucin in the small and large intestines of both mice and humans<sup>18</sup>. The entire mucus layer represents a selective microbial habitat owing to microbial adhesion via lectins and glycosidases that are expressed by only specific bacteria, and it also serves as nutrient source<sup>19,20</sup>. However, bacteria are found only in the outer layer<sup>17</sup>, probably owing to the specific

#### Mutualistic

Pertaining to a relationship between two organisms: beneficial to both organisms. The term originates from the Latin word *mutuus* (lent, borrowed or mutual).

#### Superorganism

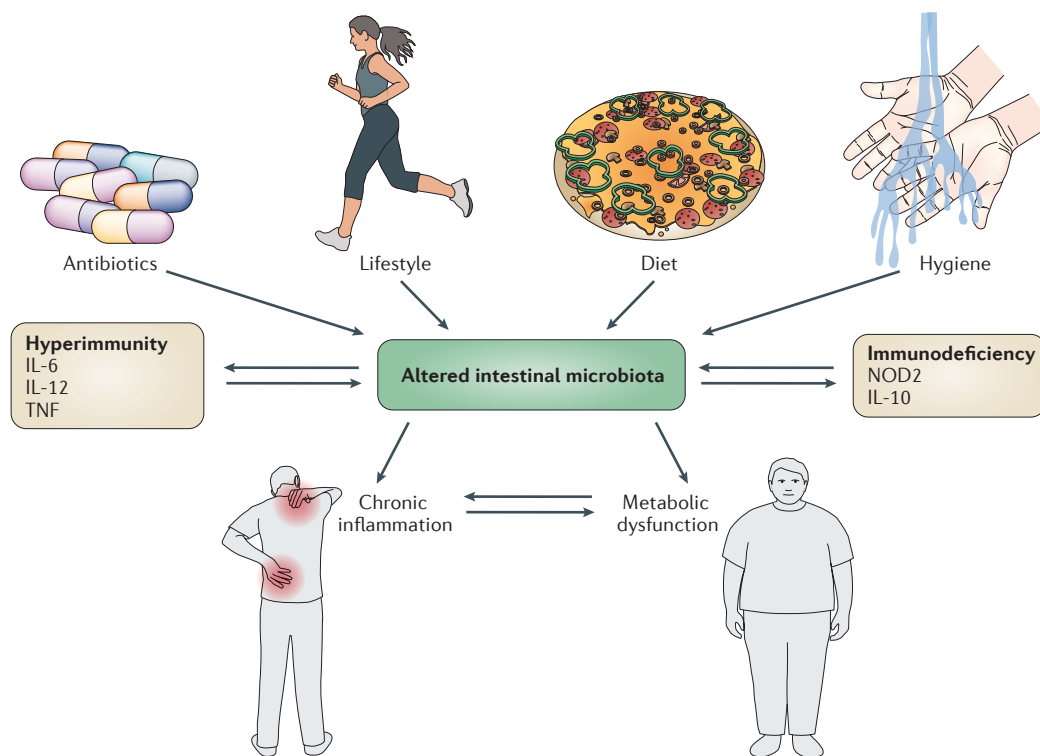
A term that extends the classical biological definition of an organism (a living system capable of autonomous metabolism and reproduction) by including the many microorganisms that live in and on that host organism, thus yielding a superior degree of complexity. The term originates from the Latin *supra* (above) and the Greek *organon* (organ, instrument, tool).

#### Symbiosis

Any close physical association between two organisms, usually from different species. This includes mutualism, commensalism and parasitism. The term originates from the Greek words *syn* (together) and *bio* (life).

#### Pathobionts

Normally harmless microorganism that can become pathogens under certain environmental conditions.



**Figure 1 | Factors shaping intestinal microbial composition and effects of dysbiosis on host health.** The composition of the gut microbiota is influenced by various environmental factors, including the use of antibiotics, lifestyle, diet and hygiene preferences. The host's genetic disposition also has a role: hyperimmunity (owing to over-representation of pro-inflammatory mediators such as interleukin-6 (IL-6), IL-12 or tumour necrosis factor (TNF)) or immunodeficiency (owing to mutations in regulatory immune proteins such as NOD2 (nucleotide-binding oligomerization domain protein 2) or IL-10) can influence the gut microbiota composition. In turn, dysbiosis affects levels of immune mediators and induces both chronic inflammation and metabolic dysfunction.

structure of the mucus layer as a whole, which is formed of interconnected sheets that create pores smaller than a bacterial cell and thus inhibit penetration<sup>21</sup>.

Comparisons of germ-free and conventionally raised animals revealed that microorganisms have major effects on mucus thickness and composition; compared with conventionally raised animals, germ-free animals have fewer goblet cells, a thinner mucus layer and also a higher percentage of neutral mucins in the colon<sup>22</sup>. Stimulation with bacterial products such as lipopolysaccharide (LPS) and peptidoglycan is sufficient to establish conventional mucus properties in germ-free mice<sup>23</sup>, but the underlying mechanisms for how the gut microbiota modulates goblet cells and mucus layer properties remain largely elusive.

Notably, *Muc2*-deficient mice or those with aberrant mucin glycosylation profiles (owing to a lack of specific glycosyl transferases) show bacterial overgrowth and either develop spontaneous colitis or are more susceptible to chemical induction of colitis, an effect that can be ameliorated by treatment with antibiotics<sup>23–25</sup>. This demonstrates the importance of the mucus layer for homeostasis in the gut and also highlights the reciprocal interaction between the mucus layer and the gut microbiota. It remains to be clarified whether disease onset in these mouse strains depends on a selectively altered and thus more colitogenic microbiota, on

mislocalization of the same microbiota or merely on increased bacterial load.

**Microorganisms induce the development of lymphoid structures.** The lymphatic system consists of a network of lymphatic vessels connecting the primary and secondary lymphoid organs. The main functions of this system are the recirculation of interstitial fluid and blood as well as the transport of lymphocytes (such as B cells and T cells) (BOX 2) and antigen-presenting cells to lymph nodes. Lymphoid tissue is classified as primary (thymus and bone marrow) and secondary (lymph nodes, Peyer's patches, tonsils, spleen and lymphoid follicles). Lymphocytes are generated in primary lymphoid tissues and are then transported to secondary lymphoid tissues, where the mature lymphocytes are exposed to antigens by antigen-presenting cells and are thus activated to initiate an adaptive immune response. The cellular interactions that occur during lymphoid tissue development and maturation are similar for both primary and secondary lymphoid organs, although the molecular frameworks differ a little (for details see REF. 26).

In addition to host genetics, several environmental factors, including contact with microorganisms, influence both the development and maturation of the immune system. The development of secondary gut-associated lymphoid tissue (GALT), such as Peyer's

## Box 2 | Lymphocyte subtypes

All lymphocytes differentiate and mature in primary lymphoid organs (the thymus and bone marrow). Mature naive lymphocytes migrate to secondary lymphoid tissues, where they become activated by antigen-presenting cells such as dendritic cells. Gut-associated secondary lymphoid tissues include Peyer's patches, mesenteric lymph nodes and lymphoid follicles<sup>139</sup>. Here, we list and describe the lymphocytes that are known to be modulated by the gut microbiota<sup>43,140,141</sup>.

### Lymphoid tissue inducer cells

(LTi cells). A unique T cell subpopulation that is characterized by the expression of ROR $\gamma$ t, CD4 and interleukin-7 receptor- $\alpha$  and the absence of CD3, B220 (an isoform of CD45) and CD11c (also known as integrin  $\alpha$ X). Their function is to recruit B cells and T cells and thereby promote the formation of secondary lymphoid tissues.

### Natural killer cells

(NK cells). Lymphocytes that recognize the abnormal antigen signatures of infected or tumour cells, which NK cells kill by lysis or apoptosis. NK cells resemble cytotoxic T cells in function but belong to the innate immune system. They express various NK cell receptors, including NKp46 (in mice; NKp44 in humans) and NKG2D. They can activate B cells and T cells and thereby stimulate an adaptive immune response.

### Natural killer T cells

(NKT cells). These cells have properties of both T cells and NK cells, as they co-express NK cell markers with a T cell receptor. NKT cells mainly recognize lipids and glycolipids presented by antigen-presenting cells via CD1d. Following activation, NKT cells produce pro-inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin-17 (IL-17). Invariant NKT (iNKT) cells are a specific subpopulation expressing an invariant T cell receptor.

### T helper 1 cells

(T<sub>H</sub>1 cells). A subset of T<sub>H</sub> lymphocytes that is characterized by the expression of interferon- $\gamma$  and transforming growth factor- $\beta$  (TGF $\beta$ ). T<sub>H</sub>1 cell differentiation is induced by contact with activated macrophages or NK cells.

### T helper 2 cells

(T<sub>H</sub>2 cells). A subset of T<sub>H</sub> lymphocytes that is characterized by the expression of the cytokines IL-4, IL-5 and IL-13. T<sub>H</sub>2 cell differentiation is induced in response to, for example, allergens and extracellular microorganisms.

### T helper 17 cells

(T<sub>H</sub>17 cells). A subset of T<sub>H</sub> cells that is characterized by the expression of IL-17, which stimulates stromal cells to express the pro-inflammatory cytokines IL-6 and IL-8, thereby attracting neutrophils and promoting inflammation to clear out invading microorganisms.

### Regulatory T cells

(T<sub>Reg</sub> cells). A T cell subpopulation that is characterized by the expression of CD4, CD25 and FOXP3 and the production of the anti-inflammatory cytokines TGF $\beta$  and IL-10. These cells can be subdivided into natural T<sub>Reg</sub> cells, which differentiate from CD4<sup>+</sup> T cells in the thymus, and inducible T<sub>Reg</sub> cells, which arise from naive T cells in secondary lymphoid tissues. Both cell types function to suppress immune activation and prevent self-reactivity, thereby reducing the risk of autoimmune disease.

### Type 1 regulatory T cells

(T<sub>R</sub>1 cells). These CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T cells are functionally equivalent to the IL-10-producing T<sub>Reg</sub> cells. They respond to microorganisms and regulate intestinal tolerance through the secretion of IL-10.

### B cells

Lymphocytes that are activated when the unique B cell receptor binds its specific antigen and that then mediate humoral immunity through the production of antibodies. B cells are also involved in lymphoid tissue organization.

**Somatic hypermutation**  
A programmed process of mutation affecting the variable regions of immunoglobulin genes during affinity maturation of B cell receptors.

patches and mesenteric lymph nodes, is initiated prenatally in the sterile environment of the fetus through induction by lymphoid tissue inducer (LTi) cells<sup>27</sup>. Briefly, mesenchymal cells are induced by retinoic acid to produce CXC-chemokine ligand 13 (CXCL13), which recruits LTi precursor cells and stimulates their clustering, leading to their maturation into LTi cells. These then

induce the differentiation of stromal organizer cells to express several cytokines and adhesion molecules that attract further immune cells, causing GALT formation<sup>26</sup>. Maturation of these tissues, including an increase in tissue size and the development of germinal centres (sites of B cell proliferation, differentiation and somatic hypermutation in lymph nodes), depends on postnatal microbial colonization<sup>28</sup> (FIG. 2). Consequently, Peyer's patches, mesenteric lymph nodes and splenic white pulp are underdeveloped in germ-free mice<sup>29</sup>.

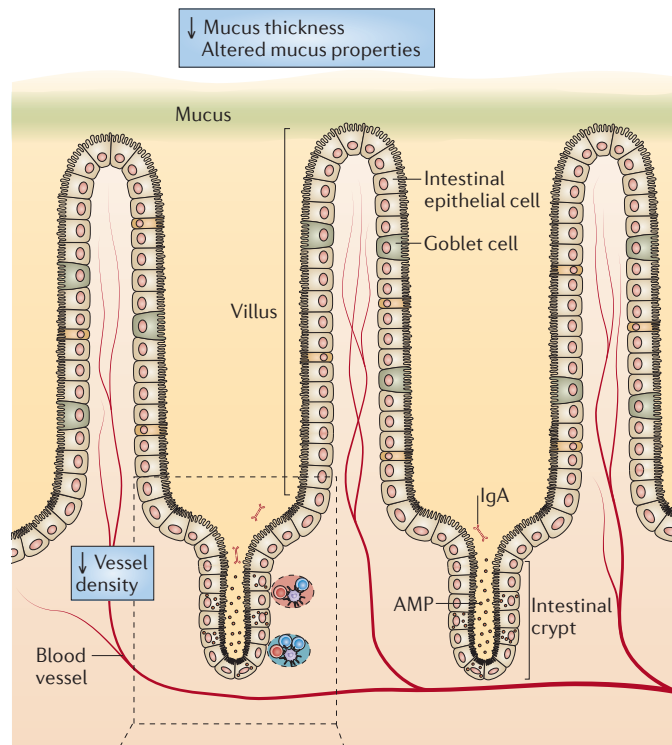
Furthermore, in parallel with microbial colonization, clusters of LTi-like cells termed cryptopatches form at birth in the connective tissue between intestinal crypts, known as the lamina propria<sup>30</sup>. Cryptopatches recruit B cells and develop into isolated lymphoid follicles (ILFs), a type of lymphoid tissue that is structurally similar to Peyer's patches and serves as an inductive site for intestinal immune reactions<sup>31,32</sup>. This process also depends on the gut microbiota, as ILFs fail to develop fully in germ-free mice<sup>33</sup>. ILF formation can be induced by exposing germ-free mice to purified peptidoglycan from Gram-negative bacteria, indicating that this process is driven solely by a specific microbial pattern<sup>34</sup>. Stromal and epithelial cells recognize the peptidoglycan of resident microorganisms mainly via signalling through the pattern recognition receptor (PRR) NOD1 (nucleotide-binding oligomerization domain containing 1) but also partially through another family of PRRs, the Toll-like receptors (TLRs). Activation of NOD1 by the gut microbiota causes increased expression of CC-chemokine ligand 20 (CCL20) and presumably also of  $\beta$ -defensin 3, both of which activate ILF formation by binding to CC-chemokine receptor 6 (CCR6) on LTi cells<sup>34</sup>.

**The gut microbiota modulates immune cell differentiation.** In addition to regulating the development of lymphoid structures, the gut microbiota has been shown to modulate the differentiation of immune cell subsets (BOX 2) and, therefore, maintain homeostatic interactions between the host and the gut microbiota.

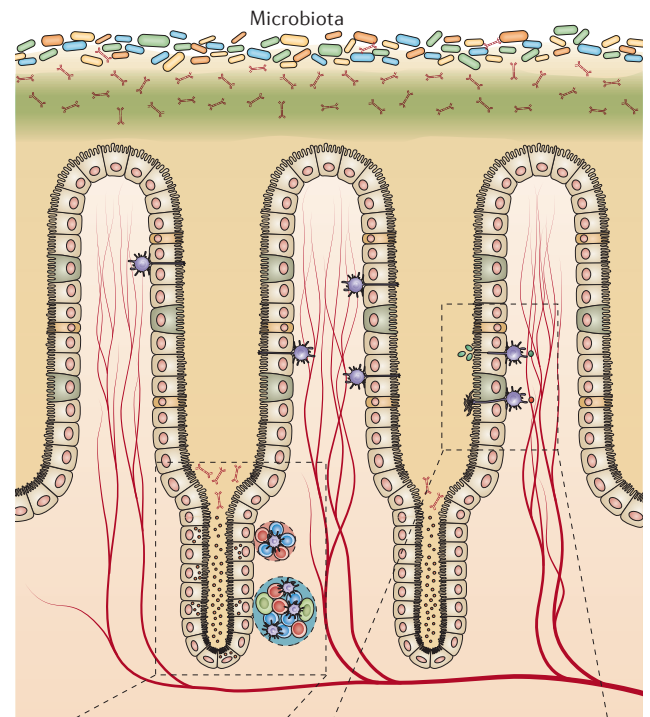
After birth, LTi-like cells that express nuclear ROR $\gamma$ t but lack NKp46 (in mice; also known as NCR1) or NKp44 (in humans; also known as NCR2) markers accumulate in both the mouse and human GALT and lamina propria<sup>35–37</sup>. Interestingly, the ROR $\gamma$ t<sup>+</sup>NKp46<sup>–</sup> LTi-like cells can differentiate into ROR $\gamma$ t<sup>+</sup>NKp46<sup>+</sup> natural killer (NK)-like cells, which differ from regular NK cells (BOX 2) in that they have intermediate expression of NK1.1 (also known as KLRB1C) and do not produce interleukin-1 $\beta$  (IL-1 $\beta$ ) or kill tumour cells<sup>36,37</sup>. This differentiation requires both IL-23, which is produced by activated myeloid cells and epithelial or endothelial cells, and the presence of the intestinal microbiota, as germ-free mice have fewer ROR $\gamma$ t<sup>+</sup>NKp46<sup>+</sup> NK-like cells than conventionally raised mice<sup>37</sup>. These cells produce IL-22, which in mice promotes the integrity of the intestinal barrier and reduces bacterial infiltration by inducing epithelial repair via signal transducer and activator of transcription 3 (STAT3) signalling and the production of antimicrobial proteins<sup>38</sup>. Thus, the normal gut microbiota promotes



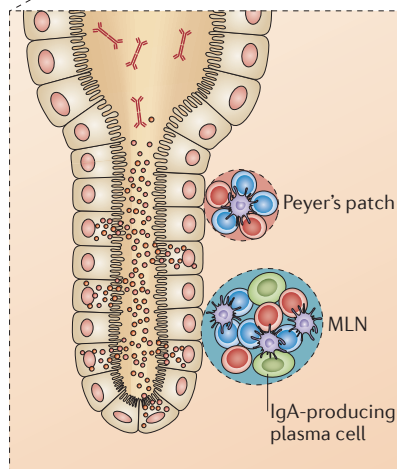
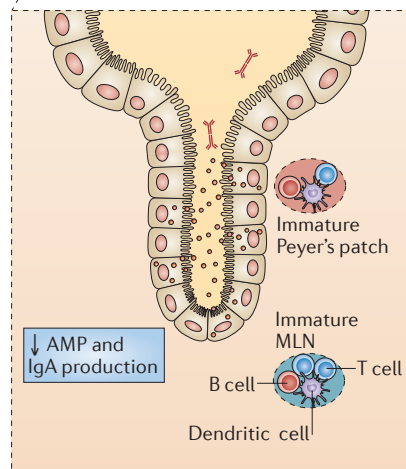
# a Germ-free mice



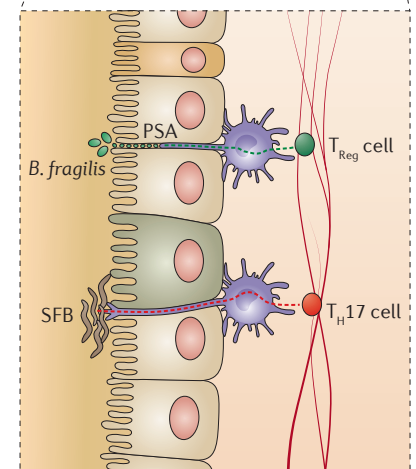
# Conventionally raised mice



# b



# c



**Figure 2 | Microbiota-induced maturation of the gastrointestinal tract.** The microbiota promotes substantial changes in gut morphology, including villus architecture, crypt depth, stem cell proliferation, blood vessel density, mucus layer properties and maturation of mucosa-associated lymphoid tissues. **a** | In germ-free mice, the villi in the distal small intestine are longer and thinner and have a less complex vascular network than the villi of conventionally raised animals. In the absence of bacteria, intestinal crypts are less deep and contain fewer proliferating stem cells. Furthermore, germ-free animals show reduced mucus thickness and altered mucus properties. **b** | Moreover, very few isolated lymphoid follicles, immature Peyer's patches and immature mesenteric lymph nodes (MLNs) are present under germ-free conditions, and levels of both immunoglobulin A (IgA) and antimicrobial peptides (AMPs) are lower than in conventionally raised animals. **c** | In conventionally raised mice, polysaccharide A (PSA) of *Bacteroides fragilis* is known to induce the expansion of CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T (T<sub>Reg</sub>) cells, which have an anti-inflammatory effect and dampen immune responses. By contrast, segmented filamentous bacteria (SFB) have been shown to induce the expansion of T helper 17 (T<sub>H</sub>17) cells, which are pro-inflammatory.

intestinal barrier function by modulating mucosal homeostasis, in part by promoting the differentiation of ROR $\gamma$ <sup>+</sup>NKp46<sup>+</sup> NK-like cells.

The intestinal microbiota also modulates the abundance of invariant NK T cells (iNKT cells), a unique T cell subset that expresses an invariant T cell receptor  $\alpha$ -chain. These cells promote inflammation, as following activation they secrete pro-inflammatory T helper 1 (T<sub>H</sub>1)- and T<sub>H</sub>2-type chemokines and cytokines, including interferon- $\gamma$ , IL-2, IL-4, IL-13, IL-17A, IL-21 and tumour necrosis factor (TNF)<sup>39,40</sup>. In contrast to the NK-like cells described above, there are more iNKT cells in the colon of germ-free mice than the colon of conventionally raised mice<sup>41</sup>, which suggests that the gut microbiota promotes homeostasis by decreasing the number of these pro-inflammatory cells. Importantly, a recent study elegantly demonstrated an age dependency of the microbial effects on the iNKT population and revealed that colonization of neonatal but not adult germ-free mice with conventional gut microbiota normalized iNKT cell numbers and protected against oxazolone-induced colitis as well as against ovalbumin-induced allergic lung inflammation<sup>41</sup>.

It has become evident that the gut microbiota shapes the T cell landscape not only in the lamina propria but also systemically and therefore modulates the homeostasis of the superorganism<sup>42</sup> (FIG. 2). Intestinal mucosal T cells are important 'legislators' of intestinal homeostasis because they not only defend against intestinal pathogens, but also promote wound healing, barrier repair and regeneration as they rapidly accumulate at sites of injury and infection<sup>43</sup>. T cells can be assigned to subpopulations that drive either a pro-inflammatory immune response (T<sub>H</sub>1, T<sub>H</sub>2 and T<sub>H</sub>17 cells) or an anti-inflammatory immune response (CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T (T<sub>Reg</sub>) cells or CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>-</sup> type 1 regulatory T (T<sub>H</sub>1) cells), depending on the cytokines that they produce<sup>44</sup> (BOX 2). The balance between both pro- and anti-inflammatory T cell subpopulations determines the overall immune equilibrium.

Interestingly, individual members of the gut microbiota have been found to drive specific T cell responses (FIG. 2c). The Gram-negative bacterium *Bacteroides fragilis* elicits an anti-inflammatory response by inducing the differentiation of CD4<sup>+</sup> T cells into T<sub>Reg</sub> cells locally in the intestinal lamina propria but also in the circulation<sup>45</sup>. T<sub>Reg</sub> cells produce IL-10 and thereby suppress the pro-inflammatory T<sub>H</sub>17 response<sup>46</sup>. This skewing event is mediated by polysaccharide A (PSA) on the outer membrane of the bacterium, which is recognized by TLR2 on CD4<sup>+</sup> T cells and activates a signalling cascade involving myeloid differentiation 88 (MYD88) to induce T<sub>Reg</sub> cell differentiation<sup>45</sup>. Indeed, a mutant strain of *B. fragilis* lacking PSA fails to initiate differentiation of T<sub>Reg</sub> cells, whereas purified PSA has the same effect as the wild-type bacterium<sup>29</sup>.

Bacteria from the Gram-positive class Clostridia have similar effects on the host immune system. A mixture of 46 Clostridia spp. belonging to clusters IV and XIVa was isolated from mouse faeces, and colonization of germ-free mice with this mixture induced the expansion of T<sub>Reg</sub> cells in the mucosal lamina propria and thereby

increased levels of the immunosuppressive cytokine IL-10 (REF. 47). Notably, compared with non-colonized germ-free mice, the Clostridia species-colonized mice were more resistant to chemically induced disruption of the gut epithelium and displayed attenuated levels of antigen-induced serum IgE. Furthermore, a similar T<sub>Reg</sub> cell response was observed when germ-free mice were colonized with altered Schaedler flora, a cocktail of eight defined bacteria including three Clostridia species<sup>48</sup>. However, the specific species in this community that are functionally responsible and the underlying molecular mechanisms of this effect are so far unknown.

In contrast to the bacteria mentioned above, segmented filamentous bacteria (SFB) elicit a pro-inflammatory immune response by promoting the differentiation of T<sub>H</sub>17 cells and, to a lesser extent, T<sub>H</sub>1 cells<sup>49,50</sup>. SFB reside in the small intestine of mice and are in direct contact with epithelial cells, which these bacteria seem to readily invade. Invasion leads to local actin polymerization in the epithelium at the interaction site, and this presumably initiates a signalling event that activates the differentiation of T<sub>H</sub>17 cells. Notably, mono-association of germ-free mice with SFB is sufficient to restore susceptibility to T<sub>H</sub>17 cell-mediated arthritis and experimental autoimmune encephalomyelitis<sup>51,52</sup>. So far, however, it is not known whether T<sub>H</sub>17 cell differentiation is induced by IEC-produced mediators, by direct interaction with antigen-presenting cells in the lamina propria (dendritic cells or macrophages) or by bacterially secreted signalling molecules (for example, metabolites)<sup>15</sup>.

**Gut microorganisms tweak the production of immune mediators.** It is clear that the gut microbiota regulates the production of cytokines and chemokines to influence the T cell repertoire of the intestine and surrounding tissue, but there is evidence that these bacteria also modulate the production of other soluble immune mediators (FIG. 2). IgA is produced by plasma cells (differentiated B cells) in the lamina propria and is transcytosed through the intestinal epithelium into the lumen, where it binds microbial antigens and thereby prevents bacterial translocation and infection<sup>53</sup>. The differentiation of B cells into IgA-producing plasma cells is induced by sensing of gut microbiota-derived flagellin via TLR5 on lamina propria dendritic cells<sup>54</sup>. IgA has a key role in barrier homeostasis, as IgA-deficient mice produce gut microbiota-specific serum IgG antibodies, indicating that there is a breach in the mucosal barrier of these mice and subsequent induction of the systemic immune system<sup>55</sup>. Furthermore, a recent study showed that microbial modulation of IgA homeostasis is in part dependent on the host protein programmed cell death 1 (PD1), which is expressed by T follicular helper cells in the germinal centre<sup>56</sup>. PD1-deficient mice harbour an altered IgA repertoire owing to changes in B cell maturation, leading to modified IgA specificity. This altered IgA repertoire then shifts the normal gut microbiota composition by reducing the numbers of bacteria from the genera *Bifidobacterium* and *Bacteroides* and increasing the number from the family Enterobacteriaceae<sup>56</sup>.

#### Experimental autoimmune encephalomyelitis

An animal model of T cell-mediated autoimmune disease in general and in particular of demyelinating diseases of the central nervous system, such as multiple sclerosis.

#### T follicular helper cells

A T cell subtype that resides in the B cell follicles of secondary lymphoid organs and expresses the B cell homing receptor CXCR5-chemokine receptor 5. These T cells mediate B cell activation and trigger the formation of the germinal centre.

In addition to IgA, the gut microbiota regulates the production of AMPs. These molecules are produced by IECs as a consequence of their tight contact with a dense and highly diverse microbial community and include defensins, C-type lectins (such as REG3 $\beta$  and REG3 $\gamma$ ), ribonucleases (for example, angiopoietin 4 (ANG4)) and S100 proteins (for example, psoriasin (also known as S100A7)), which rapidly kill or inactivate microorganisms (see REF. 57 for detailed information). Some AMPs, such as  $\alpha$ -defensins and  $\beta$ -defensin 1, are expressed constitutively<sup>58</sup>, whereas others, such as ANG4 and REG3 $\gamma$ , are induced following a microbial encounter<sup>59,60</sup>, either via PRR signalling (through TLRs and NOD-like receptors (NLRs)) or in a PRR-independent manner (for example, by microbially fermented butyrate)<sup>61,62</sup>. Furthermore, intestinal lymphocyte-derived IL-17 and IL-22, which are bacterially modulated (see above), induce the production of AMPs by IECs and Paneth cells<sup>59,63</sup>. Induction of AMPs in epithelial cells is likely to be one important mechanism for preventing breaches of the mucosal barrier and, in particular, protecting the stem cell niche in the crypts of Lieberkühn. Furthermore, AMPs not only help to sustain host–microorganism segregation, but also affect the microbiota composition. Mice that are deficient in MYD88, NOD2 or matrilysin (MMP7; a protease involved in the regulation of defensin activity), as well as mice that are transgenic for  $\alpha$ -defensin 5, have an altered microbiota owing to shifted AMP production<sup>64–66</sup>.

### Regulation of host physiology

As mentioned above, the microbiome contains >5 million genes, many of which encode biosynthetic enzymes, proteases and glycosidases, thereby greatly expanding the host's own biochemical and metabolic capability<sup>2,3</sup>. The effects of the gut microbiota on host metabolism — for example, through the microbiota metabolizing otherwise indigestible polysaccharides, producing essential vitamins and carrying out xenobiotic metabolism — have long been appreciated<sup>10</sup>. However, the effects on host physiology exceed these purely biochemical properties. In fact, the microbiota also influences a wide range of host processes and characteristics that were thought to depend solely on the genetic programme of the host, including organ development and morphogenesis, cell proliferation, bone mass, adiposity and even behaviour (FIG. 3). Below, we discuss recent advances in our understanding of the microbial modulation of these physiological properties of the superorganism.

**Development and morphogenesis.** Microorganisms affect not only the development of immune tissues, but also the development and morphogenesis of other organs and body structures in a range of species. For example, the symbiotic interaction between the fruit fly *Drosophila melanogaster* and one of its gut bacteria, *Acetobacter pomorum*, affects several host physiological properties, including developmental rate, body size, wing area, metabolism and stem cell activity<sup>67</sup>. Acetic acid produced by the pyrroloquinoline quinone-dependent alcohol dehydrogenase of *A. pomorum* triggers

*D. melanogaster* insulin signalling involving phosphoinositide 3-kinase and the forkhead transcription factor FOXO by an as-yet-unknown mechanism and thereby tunes the homeostatic programmes in the fly. The squid *Euprymna scolopes* has developed a close symbiosis with the bacterium *Vibrio fischeri*, in which the bacterium releases a tetrapeptide peptidoglycan monomer that, together with the lipid A component of LPS, is sufficient to drive the development of a light-emitting 'organ' in the squid<sup>68</sup>. Peptidoglycan signalling through a nuclear peptidoglycan recognition protein induces apoptosis, which is an integral part of light organ morphogenesis<sup>69</sup>. This organ camouflages the squid at night, as it resembles a star to predators below; remarkably, the organ is reassembled every night, as the bacteria are expelled every morning<sup>70</sup>.

In humans and other mammals, studies have shown that the intestinal microbiota has a considerable effect on the development of the gastrointestinal tract (FIG. 3). In newborns, the gastrointestinal tract is structurally and functionally immature, and maturation is induced by many factors, one them being the gut microbiota<sup>71</sup> (BOX 1). Notably, the changes in microbiota composition during weaning in both mice and humans coincide with gut maturation, indicating that specific bacteria in the pre- and post-weaning microbiota have differential effects<sup>72</sup>. The most prominent feature of germ-free animals is a greatly enlarged caecum<sup>73</sup>. Furthermore, the overall intestinal surface area in germ-free mice is reduced compared with that of conventional mice<sup>74</sup>. Germ-free mice are impaired in brush border differentiation<sup>75</sup> and have reduced villus thickness<sup>76</sup> owing to reduced cell regeneration<sup>77</sup> and a longer cell cycle time<sup>78</sup>. The number of serotonin-producing enterochromaffin cells is also higher in the gut of germ-free compared with conventional mice<sup>16</sup>, but interestingly, germ-free mice have lower levels of serotonin<sup>79</sup>, and this is thought to correlate with decreased intestinal peristaltic activity and a prolonged gastrointestinal transit time<sup>80,81</sup>. Last, microorganisms modulate epithelial permeability in the gastrointestinal tract. For example, in mice the Gram-negative bacterium *Bacteroides thetaiotaomicron* increases the resistance of the gut to injury by inducing the expression of SPRR2A, which is involved in desmosome maintenance<sup>82</sup>. Moreover, several *Lactobacillus* strains rigidify tight junctions between epithelial cells, resulting in reduced epithelial permeability<sup>83</sup>. PRR signalling was found to be important in this process, as peptidoglycan-induced TLR2 signalling in epithelial cells improves tight junction function and reduces apoptosis rates, thus enhancing barrier integrity and facilitating wound repair after injury<sup>84,85</sup>.

Interestingly, recent work has shown that, in addition to promoting gastrointestinal tract morphogenesis, the gut microbiota influences the remodelling of the vascular system<sup>76,86</sup> (FIG. 3). Colonization of the gut in germ-free mice causes restructuring of intestinal villi, which shorten and widen to prevent microbial infiltration. This restructuring increases the demand for oxygen in the gut epithelium and leads to increased amounts of sprouting endothelial cells and, consequently, angiogenesis<sup>76</sup>.

#### Crypts of Lieberkühn

Tubular invaginations of the intestinal epithelium around the villi. The crypt base contains Paneth cells, which secrete mainly antimicrobial peptides as well as other immune factors, and continually dividing stem cells that are the source of all intestinal epithelial cells.

#### Xenobiotic metabolism

The metabolism of foreign compounds that are neither produced by nor naturally found in the host, such as drugs.

#### Enterochromaffin cells

A subtype of enteroendocrine cells in the intestinal or respiratory epithelium. Enterochromaffin cells are the main source of serotonin in the body and are thereby involved in the regulation of intestinal peristalsis and nausea.

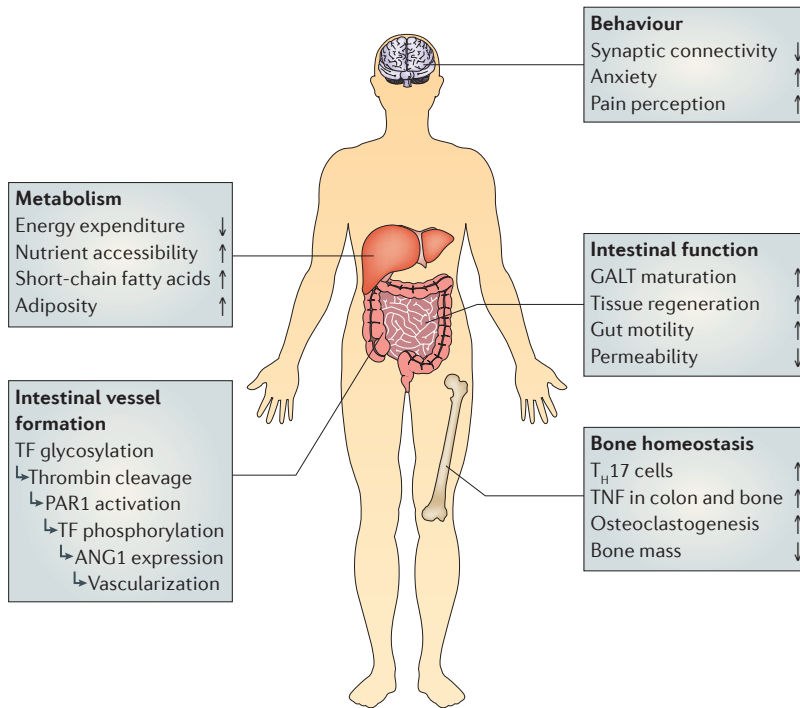
#### Desmosome

A type of junctional complex that is mainly found in epithelia (specifically, in the lateral plasma membrane of the epithelial cell) and mediates cell-to-cell adhesion to allow cells to withstand shearing forces.

#### Tight junctions

Junctional complexes that are present only in vertebrates (the invertebrate equivalents are the septate junctions) and are located at the transition of the apical and lateral membrane, closely connecting two epithelial cells and thereby making the epithelium impermeable to water and solutes.





**Figure 3 | Microbial impact on host physiology.** The gut microbiota has been shown to affect several aspects of host physiology; arrows represent either stimulatory or inhibitory effects of the gut microbiota on host physiological processes. The microbiota has been shown to influence intestinal function in the host, promoting gut-associated lymphoid tissue (GALT) maturation, tissue regeneration (in particular of the villi) and gut motility, and reducing the permeability of epithelial cells lining the gut, thus promoting barrier integrity. Similarly, the gut microbiota influences the morphogenesis of the vascular system surrounding the gut. This is associated with increased glycosylation of tissue factor (TF), which leads to cleavage of thrombin, in turn activating proteinase-activated receptor 1 (PAR1). This then phosphorylates TF to promote epithelial expression of angiopoietin 1 (ANG1), which promotes increased vascularization. Changes in the microbiota composition or a complete lack of a gut microbiota has been shown to affect metabolism, behaviour and tissue homeostasis, suggesting that the microbiota also regulates these processes. Specifically, the gut microbiota can influence the host's nervous system, decreasing synaptic connectivity and promoting anxiety-like behaviour and pain perception. In the case of host metabolism, the gut microbiota has been shown to facilitate energy harvest from the diet, to modulate host metabolism (for example, by decreasing energy expenditure) and to promote host adiposity. Finally, the gut microbiota can influence tissue homeostasis, for example decreasing bone mass by promoting the function of osteoclasts (which cause bone resorption) and increasing the numbers of pro-inflammatory T helper 17 (T<sub>H</sub>17) cells.

This process is associated with increased glycosylation and surface translocation of tissue factor (TF), leading to increased activation of thrombin. In turn, thrombin activates proteinase-activated receptor 1 (PAR1), which phosphorylates TF and promotes epithelial expression of angiopoietin 1, a protein that is required for increased vascularization<sup>76</sup>.

**Tissue and organ homeostasis.** Tissue homeostasis requires a balance between cell renewal and death, and thus a tightly regulated cell cycle, which is also modified by microorganisms. For example, in *D. melanogaster*, infection with the pathogenic bacterium *Erwinia carotovora* induces stem cell proliferation and epithelial cell renewal<sup>87</sup>. Similarly, TLR signals derived from the gut

microbiota are required for regaining tissue homeostasis following injury in the intestine in mice<sup>85</sup>. Importantly, the gut microbiota also has direct effects on tissue homeostasis, as germ-free mice have reduced epithelial cell turnover in the small intestine owing to reduced IEC proliferation, reduced crypt-to-tip cellular migration and reduced apoptosis<sup>75,88,89</sup> (FIG. 3). As the crypt contains proliferative IECs and the villus contains differentiated IECs that are in contact with the gut microbiota, these observations suggest that epithelial cells along the crypt–tip axis differ in their responses to microbial contact.

Imbalanced cellular homeostasis can result in the development of cancer, and inflammation has a crucial role in cancer initiation and progression<sup>90</sup>. Microorganisms modulate inflammation and thus could influence carcinogenesis<sup>91</sup>. Indeed, an increased bacterial load was detected in colonic biopsies from patients with colorectal cancer or colonic adenomas<sup>92</sup>. In contrast to the decreased microbial diversity that is associated with obesity and inflammatory bowel disease<sup>3,93</sup>, microbial diversity is increased in patients with colorectal adenomas<sup>94</sup>. In two mouse models of carcinogenesis, germ-free animals were protected from or showed reduced cancer development compared with conventionally raised mice<sup>95,96</sup>. Notably, bacteria are also required for the production of secondary bile acids, which have carcinogenic effects<sup>97</sup>. Some microbial species, such as *B. fragilis*, *Streptococcus gallolyticus* or *Fusobacterium nucleatum*, have been associated with cancer development<sup>98–100</sup>. This suggests that certain groups of bacteria promote, whereas others protect against, colon cancer. Therefore, selective manipulation of the gut microbiota might provide new avenues to prevent carcinogenesis<sup>90</sup>.

In addition to its effect on immune system and gut homeostasis, the gut microbiota affects homeostasis in other tissues, for example by altering bone mineral density in mice (FIG. 3). Bone remodelling occurs through the antagonistic activity of bone-forming osteoblasts and bone-resorbing osteoclasts<sup>101</sup>. Bone cells express receptors for serotonin, which, as mentioned above, is reduced in germ-free mice, and serotonin signalling inhibits bone formation<sup>102,103</sup>. Furthermore, bone loss is associated with inflammation, and T cells are responsible for bone resorption in autoinflammatory diseases<sup>104,105</sup>. T<sub>H</sub>17 cells and the pro-inflammatory cytokines TNF and IL-1 $\beta$  all promote bone resorption by inducing osteoclastogenesis<sup>105–107</sup>. Consistent with this, germ-free mice have been shown to have a higher bone mineral density than conventional mice, highlighting the fact that microbial modulation of T cell function (see above), serotonin levels and cytokine profiles might contribute to microbial modulation of bone homeostasis<sup>11</sup>. Taken together, these findings suggest that the gut microbiota can be considered an environmental factor that might contribute to osteoporosis.

**Metabolism and adiposity.** The microbiome encodes a more versatile metabolome than the host<sup>9</sup>. Although gut microbial composition differs significantly between individuals, a core microbiome can be identified,



indicating the requirement for stable functional metabolic interactions with the host<sup>108</sup>. Studies have suggested that there are differences in the gut microbiota composition between obese and non-obese individuals, although results about the gut microbiota composition in obese individuals have been conflicting<sup>109</sup>. Compared with the gut microbiome of non-obese mice, that of obese mice is enriched in genes encoding carbohydrate metabolism enzymes and was demonstrated to have a greater capacity to extract energy from the diet and to generate short-chain fatty acids<sup>110</sup> (FIG. 3). Moreover, obese humans harbour an altered microbiota with reduced diversity<sup>93,108,111</sup>, but the functional impact of this reduced diversity on the development of obesity is not yet clear. A recent report demonstrated that the gut microbiome is altered in Chinese individuals with type 2 diabetes<sup>112</sup>. Strikingly, the composition of the gut microbiota was able to predict type 2 diabetes in a second, smaller cohort.

A link between the gut microbiota and metabolism has also been demonstrated in studies using germ-free mice. These mice have reduced adiposity and require a higher caloric intake to achieve the same weight as conventionally raised mice<sup>113</sup>. This has in part been attributed to reduced energy extraction from a carbohydrate-rich diet in germ-free mice<sup>113</sup>. However, these mice are also resistant to diet-induced obesity when fed a fat- and sucrose-rich 'Western' diet containing almost no complex carbohydrates<sup>114</sup>. Thus, the gut microbiota is likely to directly modulate host metabolism (FIG. 3). For example, compared with in conventionally raised mice, the small intestine of germ-free mice has a higher expression of angiopoietin-like protein 4 (ANGPTL4; also known as fasting-induced adipose factor), which promotes fatty acid oxidation in skeletal muscle<sup>114</sup>. Furthermore, the gut microbiota might also contribute to increased adiposity and impaired glucose metabolism by stimulating inflammation and macrophage accumulation in adipose tissue<sup>115</sup>. Indeed, LPS from Gram-negative bacteria promotes hepatic insulin resistance<sup>116</sup>.

In addition to obesity, an altered gut microbiome was recently associated with symptomatic atherosclerosis<sup>117</sup>. The microbiomes of patients who have had a stroke were shown to have lower levels of carotene- and lycopene-producing enzymes and higher levels of peptidoglycan-producing enzymes than the microbiomes of individuals who have not had a stroke, suggesting that patients who have had a stroke have a more inflammatory gut milieu. Furthermore, cardiovascular disease in humans is associated with altered microbial metabolism of dietary choline<sup>118</sup>. For further details about how host-microorganism interactions can programme host metabolism, readers are referred to a recent review<sup>119</sup>.

**Effects on the brain and behaviour.** Animal behaviour and social context have been shown to shape the microbiota composition in several species, including bumblebees<sup>120</sup>, the squid *E. scolopes*<sup>121</sup> and chimpanzees<sup>122</sup>. In turn, the impact of the microbiota reaches far outside the gastrointestinal tract, also affecting behaviour (FIG. 3). For example, the gut bacterium *Lactobacillus plantarum*

modulates mate choice in *D. melanogaster*<sup>123</sup>; larval settlement of the marine tubeworm *Hydroides elegans* is regulated by the biofilm bacterium *Pseudoalteromonas luteoviolacea*<sup>124</sup>; and the composition of the human skin microbiota influences attraction for mosquitoes<sup>125</sup>, with potential consequences for disease spread. In mice, the gut microbiota modulates the levels of several signalling molecules, such as brain-derived neurotrophic factor and noradrenaline, in different areas of the brain<sup>126</sup>. Germ-free mice display an altered stress response, dysregulation of the hypothalamus-pituitary-adrenal gland axis and decreased inflammatory pain perception<sup>127,128</sup>.

To date, the best studied microbial effects are the effects on anxiety-like behaviour. Dysbiosis, as a result of either pathogenic infection or antibiotic treatment, increases anxiety-like behaviour in conventionally raised mice<sup>129,130</sup>, whereas germ-free mice show little anxiety-like behaviour<sup>131,132</sup>. The neurological defects in germ-free mice can be resolved only by colonization of neonates, indicating that there is a critical time window in which microbially induced maturation of the nervous system occurs<sup>128,131</sup>. Notably, compared with the striatum of conventionally raised mice, the striatum of germ-free mice has higher levels of the synaptic proteins synaptophysin and PSD95 (also known as DLG4), which are both involved in synaptogenesis, indicating that the gut microbiota might affect synaptic connectivity<sup>131,133</sup>.

For further discussion of microbial effects on the development of the nervous system and behaviour, readers are referred to a recent review<sup>134</sup>.

## Conclusion

Research over the past decade has accumulated a large body of evidence linking alterations in the gut microbial composition to several diseases, such as inflammatory bowel disease, asthma, arthritis, obesity and cardiovascular disease. Furthermore, it is now clear that the normal intestinal microbiota also influences numerous physiological aspects in the healthy host, including organ morphogenesis, immune system and gastrointestinal tract development and maturation, intestinal vascularization, tissue regeneration, carcinogenesis, bone homeostasis, metabolism and behaviour.

An important insight that has come from these studies is that the timing of colonization of germ-free mice seems to be crucial if these mice are to recapitulate the phenotypes of conventionally raised mice. Whereas colonization of adult germ-free mice restores adiposity to normal levels<sup>113</sup>, colonization before weaning is required to normalize behaviour and protect against the iNKT cell accumulation that is associated with asthma and inflammatory bowel disease<sup>41,131</sup>. Such early microbial colonization might have an epigenetic effect on the host through early-life imprinting, but it remains to be demonstrated how the gut microbiota achieves this. It is possible that in *Tlr2*-deficient mice, the altered microbiota contributes to altered DNA methylation patterns in cells of the colonic mucosa<sup>135</sup>. The impact of the gut microbiota as a modulator of methylation in other organs remains to be identified.

### Dysbiosis

An imbalance in the structural and/or functional configuration of the microbiota, leading to a disruption of host-microorganism homeostasis.

## Gnotobiotic

Pertaining to an organism: associated with a defined microbiota. For example, laboratory mice can be reared under sterile (germ-free) conditions or colonized with a specific collection of microorganisms. From the Greek *gnosis* (known or knowledge) and *bios* (life),

Analyses of biopsy samples obtained from gnotobiotic mouse models or from mice treated with antibiotics have been extensively used to elucidate how the gut microbiota modulates metabolic interactions and gene expression in different tissues. However, further studies are required to expand our currently limited knowledge and to establish how the gut microbiota regulates the functions of distinct cell populations in the gut.

It is important to stress that findings obtained from the study of animal models remain to be translated to diagnostic, prophylactic or therapeutic treatments for humans. One potential caveat is that microbiota members differ not only among host species but also between individual host organisms<sup>136</sup>. For example, the potent immune system-modulating SFB are found only in mice and have not yet been detected in humans. Diet is also one important factor modulating the composition of the gut microbial ecosystem<sup>136</sup>. Thus, variation in dietary habits among humans might contribute to the large inter-individual differences in the relative abundances of given microorganisms. This might constitute a major

challenge when developing diagnostic markers based on the gut microbiota. Although comparisons of gnotobiotic and conventionally raised animals are useful for identifying important physiological functions that are modulated by the gut microbiota, such comparisons cannot be automatically extrapolated to humans, and it remains unclear whether an altered microbiota associated with a disease in humans is causing, contributing to or merely a consequence of the disease state.

Immense progress has been made not only in identifying, isolating and culturing members of the gut microbiota, but also in the development of genetic tools, such as whole-genome sequencing, and in the availability of novel genetic models to dissect the interplay between the microbiome, host genetics and host physiology. Combining these tools for further studies in the upcoming years will greatly deepen our understanding of the molecular targets in the homeostatic interaction between the gut microbiota and the host, and thereby promises to reveal new ways to treat chronic inflammatory diseases and maintain health.

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## Competing interests statement

The authors declare [competing financial interests](#): see Web version for details.

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