6

Blurring the line between opportunistic pathogens and commensals

6.1 Causal reasoning 101

When Dr. Robert Koch formulated what is now known as Koch's postulates in 1884, he had a lot to fight against (Figs. 6.1 and 6.2) [1,2]. Louis Pasteur demonstrated with a swan-neck flask in 1859 that bacteria (germs) from the air ($10^{4–6}$ bacterial cells/m^3 [3], compare with the lung microbiome in Chapter 3, Fig. 3.6) spoiled gravy, instead of something that spontaneously generated in the gravy [4]. Antoni van Leeuwenhoek's early study on oral bacteria (Chapter 1, Box 1.1) also makes them sound quite benign and constitute an everyday presence. In Koch's time, there was no metagenomics, so microscopes would be the gold standard for telling this bacterium from that bacterium (Fig. 6.1), whether in a host animal or in culture. If bacteria are so omnipresent, and people did not wash their hands nearly as often as we do now, to link one bacterium to such a deadly disease as anthrax would have to pass the hardest scrutiny. And Pasteur did develop a vaccine against anthrax. The pioneering experiments by Dr. Robert Koch involved causing disease with the isolated live bacterium (or spores), and further showing that it was still that bacterium, under the microscope. Now we know that the association with the disease was unnecessarily stringent, and the effect of the intervention was too successful (Figs. 6.1 and 6.2), so well-known pathogens that have cost many lives did not fulfill Koch's postulates [2].

The biomedical field (and the finance field) has been heavily plagued by statistics from Sir. R.A. Fisher [5,6]. As physicists know very well, what really matters is not the sample size or the *P*-value, but the likely scenarios, and the probability for each scenario with the existing evidence. The probabilities are updated with new evidence (e.g., Pasteur's definitive victory over the "spontaneous generation" theory, making other explanations unlikely [4]). If a new explanation (scenario) emerges, the probabilities may need to be re-allocated among the scenarios, and some evidence may fit the new explanation better

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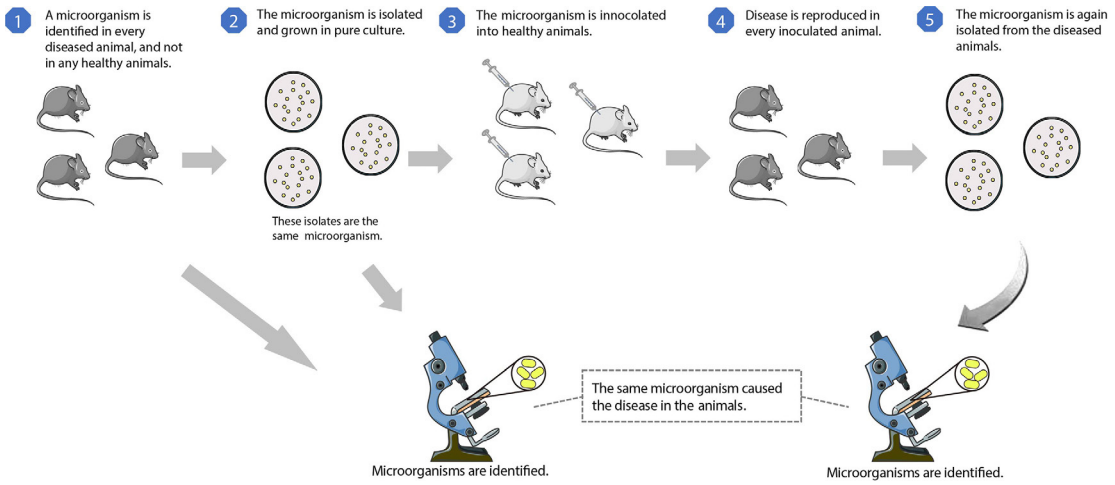

Fig. 6.1 Koch's experiment with *Bacillus anthracis*. The steps are shown according to the requirements of Koch's postulates (Fig. 6.2), and the technologies available—microscopy and isolated culture. Healthy animals are not shown, which according to Koch's postulate should not have the microorganism. Credit: Huijue Jia, Yanzheng Meng.

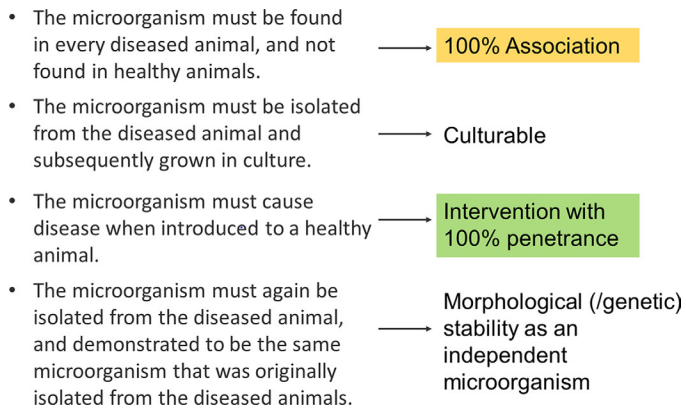

Fig. 6.2 Koch's postulates and their relevance to causal reasoning. Koch's postulates [1,2] are broken down into Association—Level 1 causal evidence, and Intervention—Level 2 causal evidence. The requirements for isolated culture and stable re-isolation are more for taxonomic and evolutionary concerns. As long as we have a proper name (and record the reference sequence used) for microorganisms identified by sequencing, cultured isolates are not relevant for causal reasoning, yet would again be useful for mechanistic and therapeutic studies. The re-isolation also addresses an alternative hypothesis that some unintended microorganism caused the same disease in the animals that were innoculated with the microorganism in question (not shown); so the disease needs to be well characterized and the laboratory animals should be under standard care to ensure reproducibility. Credit: Huijue Jia.

Box 6.1 Ockham's razor, or Newton's simplicity rule

Professor Edwin Thompson Jaynes explained in Chapter 20—"Model comparison" of his posthumous book, *Probability theory: the logic of science* [5], that Ockham's razor (Occam's razor) is intrinsic to probability theory. "Do not introduce details that do not contribute to the quality of your inferences." Formulated by the Franciscan monk William of Ockham in 1330, Occam's razor states that "Plurality should not be posited without necessity." Also known as the law of economy or law of parsimony ("It is futile to do with more what can be done with fewer").

Ockham's factor penalizes a model, essentially by considering prior information [5]. Orthodox statistical theory compares models entirely in terms of "sampling distributions." With Bayes' theorem, if the data are highly informative compared with the prior information, the relative merit of two models is determined by how high a likelihood can be attained on the respective parameter spaces, and how much prior probability is concentrated in their respective high-likelihood regions [5]. For a reasonably informative experiment, we expect the likelihood to be concentrated in small subregions, and a "simpler" model would occupy a smaller volume of parameter space, thus favored as a more plausible model [5].

Besides, Prof. Jaynes demonstrated in Chapter 7 of his book that, if we do not have any information, the Gaussian distribution is the most plausible estimate. But when we do have prior information, we should not pretend that we do not [5].

Sir Issac Newton summarized four rules of reasoning in his 1687 book *Mathematical principles of natural philosophy*. The first one—"No more causes of natural things should be admitted than are both true and sufficient to explain their phenomena."—also emphasizes simplicity and does not allow unnecessarily complicities (parameters) that do not improve the likelihood. This was also what Albert Einstein did in formulating the theory of relativity, to incorporate evidence that was not known in Newton's time.

than the old ones (Box 6.1). Of course, some people are going to be more refractory than others [5]. Back to Koch's postulates, what alternative explanations are ruled out by each experiment? Are we more and more confident with the conclusion that *Bacillus anthracis* caused anthrax (Figs. 6.1 and 6.2)?

Finding alternative explanations (hypotheses) and assigning probabilities for each explanation can depend on our own prior knowledge. For example, an analysis of vaginal microbiota community types in the Human Microbiome Project (HMP) from the United States found an association with whether the volunteers have a college degree or not (Lactobacilli and non-Lactobacilli vaginal community types) [7]. What would be the likely explanations here? (More on the cervicovaginal microbiome in Chapter 8).

"Confounders" is also a vague term from statistics that have to be abandoned [5,6]. Although there may be too many unknown factors at the beginning of a study, each scenario would still need to be clearly drawn as path diagrams in order to be mathematically workable. To try to control for (keep constant) every factor, would lead to erroneous negative associations when colliders are controlled for, and loss of

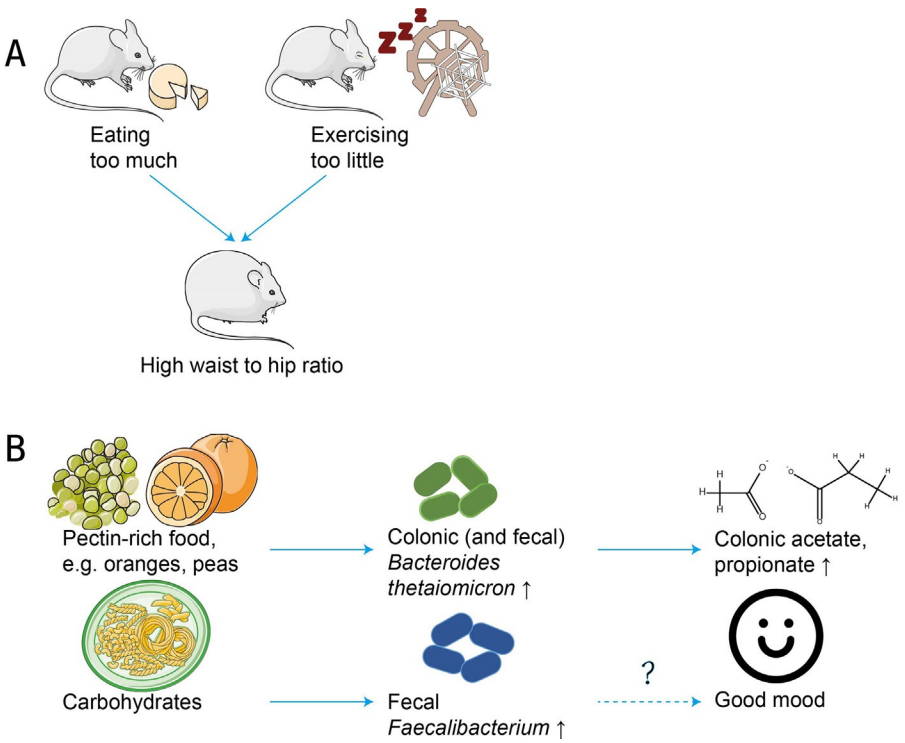

Fig. 6.3 Examples of path diagrams which would be distorted by controlling of variables, i.e., holding constant. (A) Collider. (B) On the same path. The relationship between *Faecalibacterium* and mental health is currently an association [8]. Credit: Huijue Jia, Yanzheng Meng.

effect when something in the same causal pathway is controlled for [6]. For example, if we control for fecal abundance of *Faecalibacterium* spp., we will be searching for other gut bacteria that could mediate the effect of carbohydrate (e.g., noodles) intake on a happy mood (Fig. 6.3); Or to control for tobacco use when studying lung cancer, also disabling the causal path. A more relevant example is given for a collider (Fig. 6.3), other than the famous examples of "handsome but mean guys"—apparent negative association because the mean and not handsome guys had zero opportunity, or talents and good look in actors and actresses [6]. If we all agree that eating too much or exercising too little can both make one fat (waist-hip ratio (WHR) here, Fig. 6.3), controlling for WHR or BMI (Body mass index, weight/height^2) in analyses would create a negative association between eating too much and exercising too little, i.e., an apparent positive association between eating and exercising because controlling for a collider (constant waistline or weight) opens the door there. So we are happily in the loop of eating, exercising, and more eating, while trying to maintain the same shape.

Path diagrams provide a transparent common ground for subsequent investigations, by outlining the working hypotheses [5,6]. For example, are we measuring the necessary hormones when we talk about differences in the microbiome of infants delivered by Caesarean section versus vaginal delivery, or preterm versus term birth (Fig. 6.4; More on infants in Chapter 8)? In addition to a role in the development

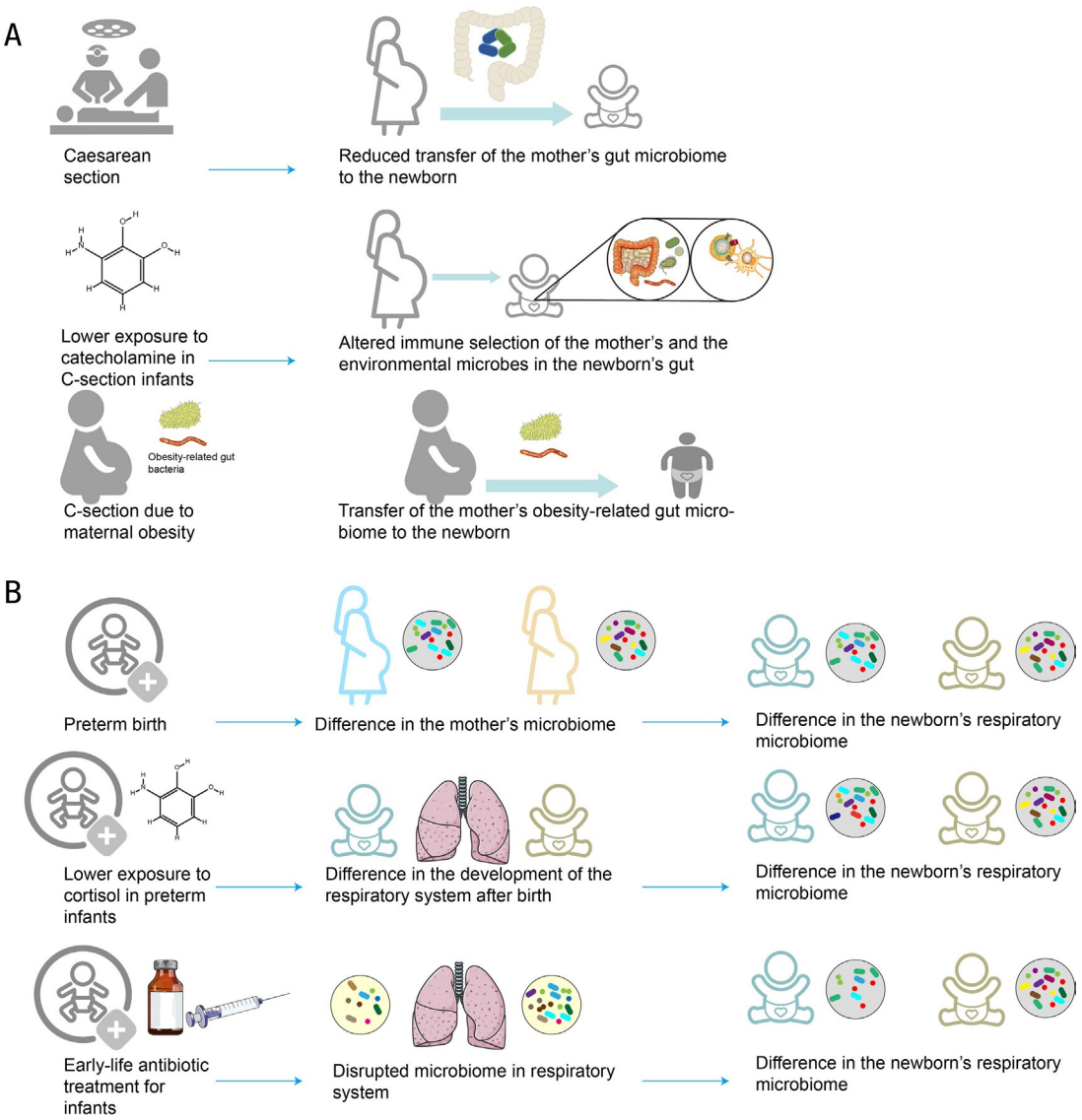

Fig. 6.4 Examples of alternative path diagrams that could potentially explain differences in the infants' microbiome. (A) A few examples for C-section. (B) A few examples of preterm birth. Inspired by [9]. Credit: Huijue Jia, Yanzheng Meng.

Worked sample 6.1

A recent publication from the American Gut project (16S rRNA gene amplicon sequencing) controlled for alcohol consumption as well as dietary information, and lost most of the fecal microbiome signal for T2D [12]. Dietary information comes down to ingredients such as amino acids, vitamins, fibers [13], sugars, lipids, and additives (e.g., Ref. [14]). Alcohol is epidemiologically known as a protective factor for rheumatoid arthritis [15]. MHC class I expression is upregulated in peripheral blood lymphocytes during acute ethanol intoxication [16]. Ethanol and its metabolite acetate (among the SCFAs, Fig. 6.5 in Section 6.3.1), potently modulate the function of T follicular help cells (T_{FH}) [18], and acetate induces IgA (immunoglobulin A) production [19].

Without getting lost in the details, how would you draw a path diagram here to interpret the chain of events? Which path do you think is more important to a T2D population you are more familiar with? What other evidence would you look for?

of the respiratory system [9], corticosteroids from the mother's milk have been shown in rats and mice to prime the hypothalamic-pituitary adrenocortical (HPA) axis [10], which would be central to stress responses [11]. New paths can always be added for a more comprehensive or more precise picture. When sailors only knew that lemons prevent scurvy, the lemons could be boiled (which destroys vitamin C) and people would no longer believe that the lemons have an effect [6].

6.2 Levels of existing evidence for the human microbiome and diseases

Numerous associations have been reported between taxa in the human microbiome and diseases, and between taxa in the microbiome and circulating molecules that are relevant to diseases, e.g., cytokines, lipids, amino acids. Phylum level claims tend to be affected by the compositional nature of relative abundance data [20]. For example, Firmicutes and Bacteroidetes almost add up to 1 in the mouse gut, so they appear negatively correlated, and whichever disease that enriched for Firmicutes would also show up as depleted for Bacteroidetes. When there are hundreds of taxa in a more even distribution (e.g., not like the vaginal samples with > 90% Lactobacilli), this is less of a problem [20,21].

Besides the associations, some of the microbes' roles in diseases are reaching Level 2 evidence—(Randomized) intervention (Box 6.2), which adds the exposure to see an effect, or Level 3 evidence—Counterfactual, which removes the exposure in one's mind to assess causality (Table 6.1), something AI (artificial intelligence) cannot do.

The readers are encouraged to try to read the most layman book from Prof. Judea Pearl, *The book of why* [6], with plenty of examples from other disciplines [5,6]. Without a randomized controlled trial (RCT), mendelian randomization (MR) could also provide Level 2 causal evidence (Box 6.1) [22,26], using human genetic information (summary statistics, quantities for a population that can be analyzed without access to individual genetic data) from large cross-sectional cohorts, or having all the measurements in the same cohort (one-sample MR versus two-sample MR). Intervention experiments on mice are usually not randomized, but unlike human cohorts, we have no specific reason to believe that the assignment of this mouse and that mouse into experimental groups is affected by some phenotype of the mice (e.g., a

Box 6.2 Mendelian randomization (MR)

A common problem with interventions is that other factors may be affecting the exposure X, when we try to look at the effect of X on the outcome Y. For example, taking yogurt every day may say something about one's job, education, economic status, which may also correlate with going to the gym on a regular basis. This is not to discourage longitudinal cohorts, which have their unique values if painstakingly followed for decades, and would help with counterfactuals.

Randomized controlled trials (RCTs) assign the value of X in a random manner, thereby severing its link with other factors, e.g., taking yogurt or not have nothing to do with the other things mentioned above, and now we can more confidently see whether there is a beneficial effect of yogurt on cardiovascular health, gastrointestinal health, etc.

MR could have the same effect of removing X from other factors, when looking at the effect of X on Y. Here, the randomization is based on the naturally random assortment of parental alleles into daughter cells during meiosis. This is typically analyzed with multiple SNPs (single-nucleotide polymorphisms). The SNPs (referred to as an instrumental variable) have to associate with the exposure X, to together make an effective instrumental variable that explains a considerable portion of the variance in X (e.g., > 20%), otherwise it may be no surprise that we could not see an effect of X on Y. The instrument variable should not influence the outcome Y without going through the exposure X. And the instrument variable should not associate with another factor that influences both X and Y [22], which might then be an actual cause, instead of a "confounder."

MR can be very powerful in discovering causal relationships, including where RCTs are ethically impossible. For example, through two-sample MR combining the SNPs-microbiome association from the 4D-SZ cohort from China and the SNPs-diseases association from Biobank Japan (BBJ), gut *Streptococcus parasanguinis* has been shown to contribute to the heart problem of posterior wall thickness, as well as colorectal cancer, raising the level of causal evidence above the MWAS associations [23–25] (Table 6.1). Higher BMI (body mass index), smoking, and coffee consumption were shown by MR to increase rheumatoid arthritis (RA), while iron, linoleic acid (a major polyunsaturated fatty acid (PUFA) that could stimulate testosterone synthesis) and years of education were protective [26].

When interpreting results from large cohorts, it is important to bear in mind that we are limited by the phenotypes and questionnaires collected, so a causal signal could still mean another unasked question that strongly correlates with the available one, at least potentially on the same path.

Table 6.1 Examples of causal evidence for the microbiome.

Level of causal evidence		Evidence	Reference
1	Association	Fecal *Escherichia coli* associated with GLP-1 level; Fecal *Escherichia coli* enriched in prediabetic patients compared to controls	[27,28]
1	Association	Fecal *Escherichia coli* enriched in atherosclerotic cardiovascular disease patients compared to controls, and negatively associated with hand grip strength, a known epidemiological factor for cardiovascular events	[24,29]
1	Association	Fecal *Bacteroides caccae* enriched in T2D patients[a]	[28,30]
1	Association	Fecal *Eggerthella* enriched in (pre)diabetic patients; and associated with early frailty	[28,31,32]
1	Association	Fecal *Ruminococcus torques* enriched in ulcerative colitis patients; and associated with loose stool according to BSS	[33,34]
1	Association	Smoking associated with oral *Veillonella* and *Prevotella*	Section 4.4.1
1	Association	Oral *Lachnoanaerobaculum umeaense* and two *Oribacterium* species associated with serum urate level, and with SNP in the uric acid transporter gene *SLC2A9*	[35]
2	Intervention (Randomized controlled trials (RCT))	A multicenter RCT for *Lactobacillus* and *Bifidobacterium* (a 9-strain mix of *B. longum*, *B. breve*, *L. casei*, *L. crispatus*, *L. fermentum*, *L. plantarum*, *L. rhamnosus*, *L. salivarius,* and *L. gasseri*) together with berberine treatment (after the antibiotics gentamycin sulfate) did not result in better blood glucose level in T2D patients, yet showed some effects on lipids	[36]
2	Intervention (RCT)	RCT for *L. crispatus* CTV-05 after metronidazole treatment decreased recurrence of bacterial vaginosis within 12 weeks (46/152 recurrence with treatment versus 34/76 recurrence with placebo)	[37]
2	Intervention (Mendelian randomization (MR)	A fecal Lachnospiraceae species showed bidirectional increase with serum urate level; Fecal microbiome pectin degradation module (e.g., from *Bacteroides*, *Fusobacterium*) appeared to increase serum urate level	[25]
2	Intervention (MR)	Fecal *Escherichia coli* could lead to T2D, heart failure, colorectal cancer, etc.	[25]
2	Intervention (MR)	Fecal *Streptococcus parasanguinis* could lead to a thicker posterior wall in the heart, and colorectal cancer	[25]
2	Intervention (MR)	Fecal Saccharibacteria (TM7) could decrease serum creatinine and increase the estimated glomerular filtration rate (eGFR)	[38]

Table 6.1 Examples of causal evidence for the microbiome—cont'd

Level of causal evidence		Evidence	Reference
3	Counterfactual	If there is no HPV (human papillomavirus), there will be no cervical cancer	Free insurance can be provided with every test
3	Counterfactual	If there is no *Helicobacter pylori*, will there be no gastric cancer? Will there be no gastric ulcer?	
3	Counterfactual	If there is no *Porphyromonas gingivitis*, *Treponema denticola*, *Tannerella forsythia*, and *Prevotella intermedia*, will there be no periodontitis?	

This is not meant to be an exhaustive list, and the associations are especially numerous. It serves to illustrate the three levels of causal evidence as was summarized by Pearl and Mackenzie [6]. GLP-1, glucagon-like peptide 1 (see also Section 6.3.3). BSS, Bristol stool scale, is mentioned in Chapter 3. SNP, single-nucleotide polymorphism.
[a]Other Bacteroides species reported in other studies, e.g., Refs. [31,39]; see also Section 6.3.
Credit: Huijue Jia.

fatter mouse is more cooperative to an intervention?). However, germ-free mice are not normal in metabolic, immunological, and neurological states [40–42]. The microbiome is also much different between SPF (specific-pathogen free) mice and humans, and between mice facilities [43,44], both regarding the species and the interactions.

Intuitively, microbial associations involving well-established molecules that contribute to disease are considered as stronger evidence as mere association with disease cases compared to healthy controls (Table 6.1). Having more associations along the same line indeed greatly reduces the likelihood that we have just caught one spurious association when we do so many statistical tests (Professor Jaynes's book [5] may be mathematically intimidating for many readers, but one can safely skip some equations). M-GWAS (Metagenome-genome-wide association studies) associations with human genes are regarded as perhaps more than Level 1 association (Table 6.1), as the established link between a gene and a disease adds to the credibility of a new association between a microbe and a disease, narrowing down the parameter space and pointing at experiments to do. The beauty of Guilt-by-association is, we are not arbitrarily following a hypothesis that may turn out to be a small question in the big picture, and to have an association is much better than nothing.

Infection by the gastric pathogen *Helicobacter pylori* is typically eradicated in developed countries, while *H. pylori* continues to colonize about 50% of the world's population [45]. Only 1%–3% of *H. pylori*-infected individuals would develop gastric cancer, depending

on genetic features of the *H. pylori*, possibly other microbes (e.g., *Lactobacillus* may be protective) [46], as well as the site of *H. pylori* colonization, host genetics and immune responses [45,47]. As the strongest risk factor for duodenal and gastric ulcer, and gastric cancer, *H. pylori* has not reached Level 3 causal evidence, not because of the less than 100% disease manifestation (Koch's postulates, Fig. 6.2) which just means that other factors are also part of the path diagram, but because we cannot confidently make a counterfactual (Table 6.1), and tell people that they would not get gastric cancer within the next few years. It would still be a counterfactual if it turns out that we have to exclude some rare form of gastric cancer that is not caused by *H. pylori*. Gastric cancer patients are not routinely examined for *H. pylori*; It is likely that for some patients, eradication or spontaneous elimination (e.g., by increased Lactobacilli with coffee consumption? This is rather anecdotal at this point.) of *H. pylori* was too late, and no additional intervention has been developed to stop further deterioration of the gastric epithelium. This would need to be better worked out.

The periodontitis-promoting bacteria are usually dislodged mechanically or killed chemically (e.g., mouthwash targeting *Porphyromonas gingivalis*). If the answer is yes to the counterfactual question regarding their causal role in periodontitis (Table 6.1), what would be the consensus for best practice? Do the bacteria have some good functions for host immunity (e.g., in defense of a pathogen that is no longer of concern) or for host metabolism that we need to keep an eye on (e.g., salivary *Porphyromonas* correlated with a high copy number of the amylase gene in the human genome [48])? Or do the chemicals used for oral hygiene have side effects from the microbiome point-of-view? In addition to periodontitis and dental caries, what would be the recommendation if there are too many colorectal cancer, colitis, or liver disease-promoting bacteria in the oral microbiome (e.g., Refs. [49–51]), although not yet in the feces?

As a community of microbes, more convoluted scenarios can take place. Some microbes modify the habitat or provide a common good (nonspecific cross-feeding), thereby affecting many other microbes

Worked sample 6.2

What complications do you think multicenter RCTs intend to control for, that is of concern in single-center RCTs?

If the participants tend to toss away what they are given (e.g., 50% chance of placebo, 50% chance of the bacterial formulation being trialed), can you collect metagenomic samples to double-check that they are taking the formulation?

For how long can the participants be followed after taking the bacterial formulation? Do you expect cell phone applications or other new technologies to help? (Also for Chapter 8).

(Chapter 2). For example, *Lactobacillus* and *Bifidobacterium* can reduce nitrite into nitric oxide, which impacts blood flow and motility, while ridding the host of the potential carcinogen, nitrite [52] (worth considering for the comprehensive picture of *H. pylori* and gastric cancer). *Ruminococcus gnavus*, a common bacterium enriched in Crohn's diseases, can hydrolyze the blood group B glycan into blood group O [53], degrade mucin glycans in a way different from other commensals [54], and secrete a complex glucorhamnan polysaccharide that induces inflammation through TLR4 (Toll-like receptor 4) [55]. In gnotobiotic mice, it has been shown that sialidase from *Bacteroides thetaiotaomicron* liberates sialic acid from the gut mucosa, which is required for the expansion of pathogens such as *Salmonella typhimurium* and *Peptoclostridium difficile* (renamed from *Clostridium difficile*) [56]. Vaginal *Gardnerella vaginalis* can express sialidase; *Prevotella bivia* encode sulfatase and sialidase that all damage the healthy mucus layer and promote a dysbiotic (imbalanced) microbiome on the way to bacterial vaginosis, and with more steps, preterm birth [57,58]. Intestinal *Candida albicans* is a major fungal inducer of T helper 17 (Th17) cell response, which would cross-react to fight against other fungi, such as airway inflammation due to *Aspergillus fumigatus* [59]. In animal models, the dose of *Staphylococcus aureus* pathogenesis was lowered by the co-presence of *Staphylococcus epidermis*, *Micrococcus luteus* or *M. luteus* cell wall peptidoglycan, due to reduced oxidative bursts of liver Kuffer cells in the presence of the commensals [60]. Some of the currently single-microbe conclusions may also turn out to be path diagrams with more branches. We will eventually understand each path, the probability in each path, and make informed decisions for each patient (Chapter 7).

6.3 From microbes to molecules

In analogy to SARS-CoV-2 causing COVID-19, a systematic understanding of the pathogenicity would need to include everything from human and microbial genetics to etiology of the complex disease. Multiple molecules from one microbe could all contribute to its pathogenic or beneficial role in disease.

6.3.1 Multiple effective molecules from *Akkermansia muciniphila*

Akkermansia muciniphila inversely correlated with body weight [61], fecal salinity [62], increased in mice model of Roux-en-Y gastric bypass (RYGB) [63], and in T2D patients treated with metformin [64]. An outer membrane protein Amuc_1100 from *A. muciniphila* signaled through Toll-like receptor 2 (TLR2), and was partly responsible

for the beneficial effect of pasteurized (heat-killed) *A. muciniphila* [65]. A single-center RCT has been completed on 32 volunteers using pasteurized *A. muciniphila*, showing significantly better insulin sensitivity, and some difference in body weight, fat mass, and hip circumference [66]. On the other hand, exposure to cold temperature decreased *A. muciniphila* relative to some of the *Firmicutes* [67], while *A. muciniphila* increased thermogenesis [68]. This function has recently led to the discovery that the bacterium could secrete a protein called P9, which induced GLP-1 (glucagon-like peptide-1) in circulation to promote glucose homeostasis, and induced uncoupling protein 1 in brown adipose tissue for thermogenesis [68]. *Akkermansia* and many other bacteria produce acetate (a major SCFA, Fig. 6.5), which just as vinegar does, can increase appetite [69], as well as promoting T follicular help cells (T_{FH}) [18], and inducing more IgA [19]. Fecal propionate, however, was reported by one MR study to promote Type 2 diabetes [70]; yet this might reflect pleiotropic effects of diabetes-promoting *Bacteroides* and *Prevotella*, which produce propionate (please continue reading to Sections 6.3.2 and 6.3.3), or less succinate production by *Bacteroides* and *Prevotella* for intestinal gluconeogenesis [71]. In chicken, duodenal *A. muciniphila* abundance associated with feed efficiency; so did cecal abundance of *Parabacteroides*, *Lactobacillus*, *Corynebacterium*, etc. [72].

As mentioned in Chapter 2, *Akkermansia muciniphila* has also been associated with diseases such as colorectal cancer [73,74], atherosclerotic cardiovascular disease [24], Alzheimer's disease, and schizophrenia [75,76]. The disease associations may involve some different genomic features of the bacterium [77,78], access to some different host molecules (e.g., mucin proteins other than Muc2 expressed in cases of infection and cancer [79]), circadian rhythm (Chapter 2, Box 2.5) or some other mechanisms.

Back to the membrane proteins, T cell-interacting peptides from both Amuc_RS03735 and Amuc_RS03740 of *Akkermansia muciniphila* specifically induced IgG1 (immunoglobulin G1), instead of the more common IgA in the intestine [80]. Such immune modulation raises the stakes of supplementing the bacterium for more effective PD-1 checkpoint immunotherapy.

6.3.2 Branched chain amino acids for muscles and diabetes

All clades of the *Prevotella copri* complex produce branched chain amino acids (BCAAs) [81]. In this case, even though bacteria other than *Prevotella copri* also contribute to the production and metabolism of BCAAs, *Prevotella copri* makes a causal contribution to BCAAs, and consequently susceptibility to diseases that have been established for BCAAs.

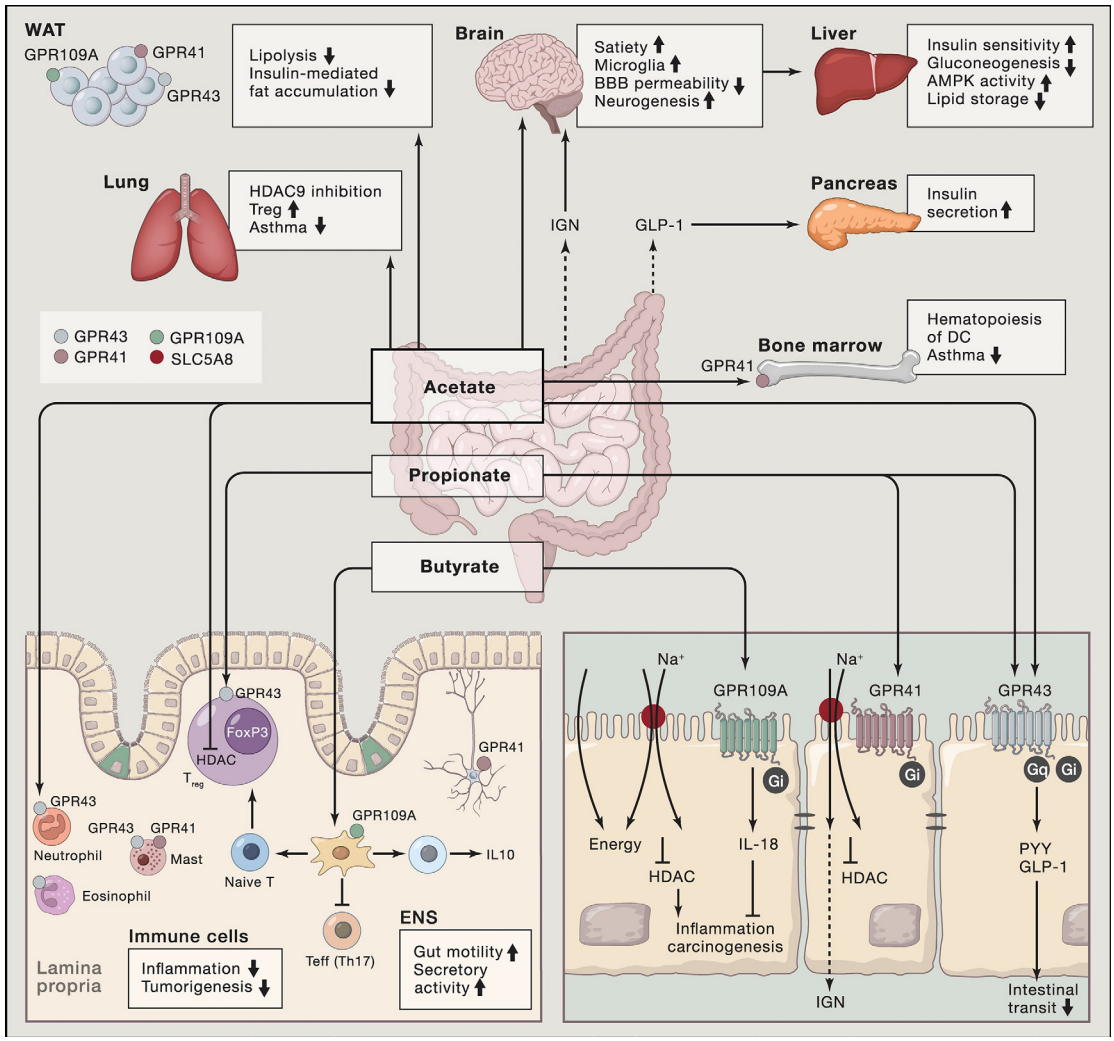

Fig. 6.5 A summary of physiological functions of microbially produced acetate, propionate, and butyrate (SCFAs), including the human receptors. Fermentation of dietary fiber leads to the production of SCFAs via various biochemical pathways. The size of the letters symbolizes the ratio of SCFAs present. In the distal gut, SCFAs can enter the cells through diffusion or SLC5A8-mediated transport and act as an energy source or an HDAC inhibitor. Luminal acetate or propionate sensed by GPR41 and GPR43 releases PYY and GLP-1, affecting satiety and intestinal transit. Luminal butyrate exerts antiinflammatory effects via GPR109A and HDAC inhibition. Furthermore, propionate can be converted into glucose by IGN, leading to satiety and decreased hepatic glucose production. SCFAs can also act on other sites in the gut, like the ENS (enteric neural system), where they stimulate motility and secretory activity, or the immune cells in the lamina propria, where they reduce inflammation and tumorigenesis. Small amounts of SCFAs (mostly acetate and possibly propionate) reach the circulation and can also directly affect the adipose tissue, brain, and liver, inducing overall beneficial metabolic effects. Solid arrows indicate the direct action of each SCFA, and dashed arrows from the gut are indirect effects. For diseases, also see [17]. Credit: Fig. 4 of Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 2016;165:1332–345. https://doi.org/10.1016/j.cell.2016.05.041.

T2D is a major disease downstream of BCAAs in the middle-aged and elderly population [39,82,83]. At a young age, BCAAs are good for muscle growth [84,85]. Differences in BCAA content between plant and animal foods contribute to the beneficial effect of low-protein or vegetarian diets [86]. In addition to functions in muscles, heart, adipose tissues, and liver, activation of the mammalian target of rapamycin (mTOR) pathway by BCAAs are required for maintenance of Treg (regulatory T) cells, which are in a high metabolic status [87], a potential explanation for the link between socio-economic status and autoimmune diseases such as RA.

Like the popular reference to "SCFAs," BCAAs may be another simple abbreviation that obscures differences between the molecules. Reducing isoleucine or valine, but not leucine, has recently been shown to improve metabolic health, and dietary level of isoleucine associated with BMI [86].

6.3.3 Molecular mimicry of autoantigens

Besides *Prevotella copri*, the other major "enterotype" (Chapter 2) *Bacteroides* spp. have been associated with Type 1 and Type 2 diabetes, IBD, and colorectal cancer [28,30,31,39,74,88–90]. In addition to SCFAs, BCAAs, and LPS (lipopolysaccharides) structure [91], antigenic properties of *Bacteroides* spp. proteins have also been identified. The integrase (an enzyme carried by transposable elements to integrate into the genome) in species such as *Bacteroides vulgatus* and *Bacteroides dorei*, contain a low-avidity mimotope of the pancreatic β cell autoantigen islet-specific glucose-6-phosphatase-catalytic-subunit-related protein (IGRP, amino acids 206–214), which promotes recruitment of diabetogenic cytotoxic T cells to the gut, and suppresses colitis in an MHC-I (Major histocompatibility complex I) dependent manner [92] (Fig. 6.6). For some people, pancreatic β cells may be expressing too much of the susceptible MHC-I that pancreatic β cells, instead of autoantigen-loaded dendritic cells (DC) are killed in the pancreatic lymph node (PLN).

Another peptide from an outer membrane polysaccharide utilization (PUL) protein of *Bacteroides thetaiotaomicron* (BT4295, amino acids 541–554) was recognized by T-cell receptors (TCRs), without known mimicry, and also contribute to protection against colitis through Treg cells in a mouse model [93]. BT4295 is incorporated into outer membrane vesicles (OMVs), and BT4295 expression is downregulated by dietary glucose [93]. The *Bacteroides* "enterotype" may need to be further divided into subtypes such as *Bacteroides thetaiotaomicron* versus *Bacteroides uniformis*, for potentially different susceptibility to inflammation and metabolic diseases.

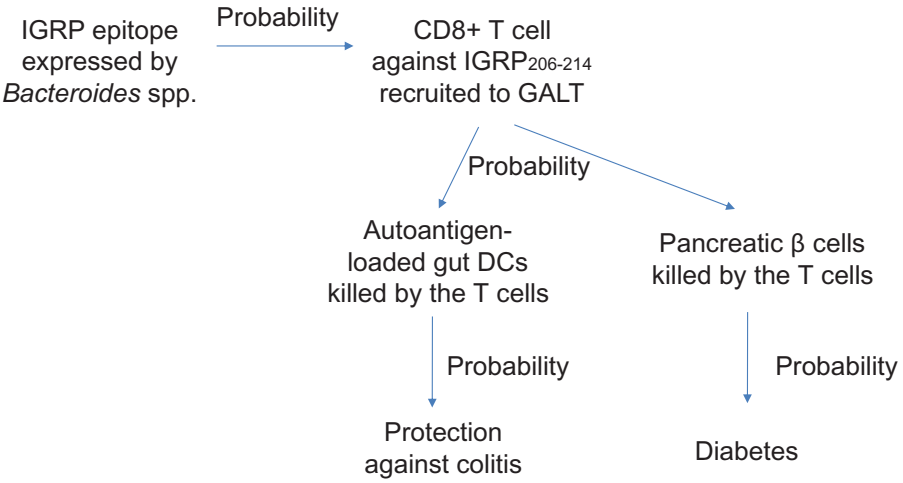

Fig. 6.6 Working model in path diagrams for the autoantigen in *Bacteroides* integrase. Summarized from [92]. There is a probability for each step, depending on the cell populations and their evolutionary history. Some people may never get the disease. Credit: Huijue Jia.

Molecular mimicry of the gut and oral microbiome to self-antigens has been tentatively explored for RA, ankylosing spondylitis, and Sjogren's syndrome [94–96]. For systemic lupus erythematosus (SLE), microbiome from multiple body sites of both healthy adults and SLE patients contain homologous sequences that map to the autoantigen Ro60 [97]. Patient T cells that react to the Ro60-mimic from skin *Propionibacterium propionicum* and from gut *Bacteroides thetaiotaomicron* showed some activity against human Ro60; Patients T cells against human Ro60 also cross-reacted with *Propionibacterium propionicum* [97].

6.3.4 Other examples, outer membrane vesicles, phages

One explanation for the enrichment of *Escherichia coli* in prediabetic individuals (Table 6.1) is its production of indole, which stimulates enteroendocrine L cells to produce glucagon-like peptide-1 (GLP-1) and therefore insulin secretion by pancreatic β cells [98]. So the increase of *Escherichia coli*, together with *Akkermansia muciniphila*, in the mice model of RYGB [63] is likely central to the effect of the surgery in treating metabolic syndrome.

This is of course a very versatile bacterium with many other functions. In pancreatic cancer, cytidine deaminase (CDD_L) encoded by *Escherichia coli* could metabolize the chemotherapeutic drug gentamicin (2′,2′-difluorodeoxycytidine) (Fig. 6.7) [99]. Such in vitro experiments after bioinformatic analyses are very useful for our understanding of individual members of the microbiome.

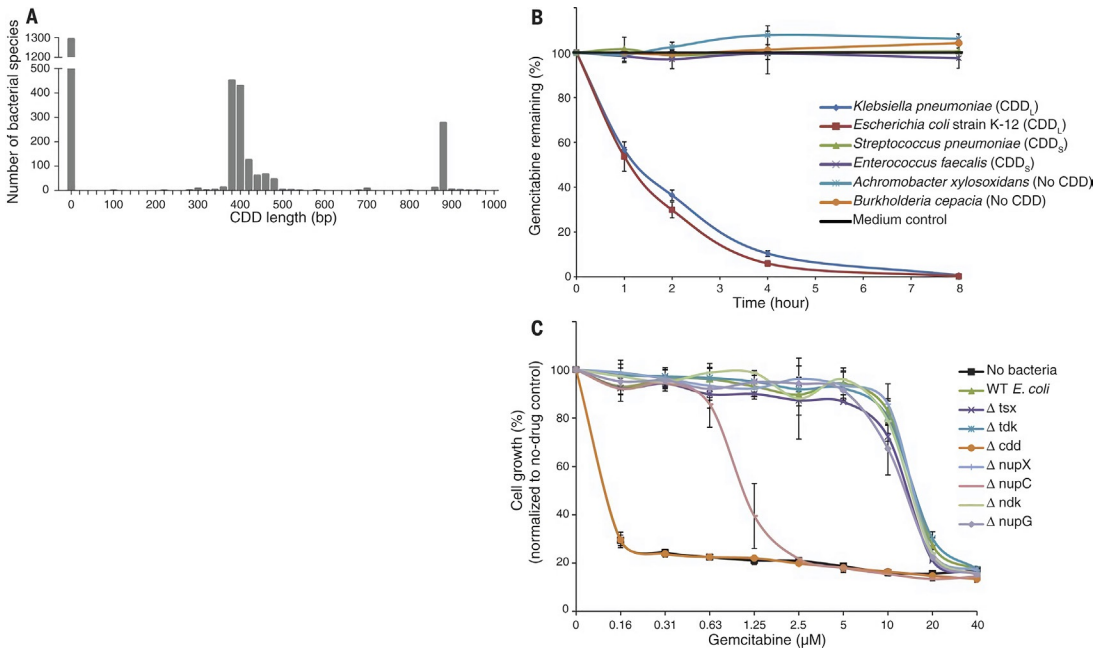

Fig. 6.7 Experiments to show that the long isoform of intestinal bacterial cytidine deaminase (CDD) mediates gemcitabine metabolism. (A) Histogram of CDD DNA sequence length across all bacteria in the KEGG database. bp, base pair. (B) Gemcitabine (4 mM) was incubated with 107 bacteria in an M9 minimal salt medium. Bacteria were filtered from the media at different time points, and the remaining gemcitabine was detected by HPLCMS/MS. Bars represent the standard deviation between two biological replicates, each containing two technical repeats. (C) WT (wild-type parental) *Escherichia coli* strain K-12 (long CDD), CDD knockout (delta) strains of the parental *Escherichia coli*, and bacteria-free media were each incubated with different gemcitabine concentrations for 4 h. Bacteria were then filtered out, and the flow-through media were added to GFP-labeled AsPC1 human pancreatic adenocarcinoma cells. The growth of AsPC1 cells after 7 days, as measured by GFP, was normalized to a no-drug control. Bars represent the standard deviation between four replicates. Credit: Fig. 2 of Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017;357:1156–160. https://doi.org/10.1126/science.aah5043.

In the lung, outer membrane vesicles (OMVs) from *Bacteroides ovatus*, *Bacteroides stercoris*, and *Prevotella melaninogenica* could induce expression of the cytokine interleukin-17B (IL-17B) by alveolar macrophages and promote lung fibrosis [100].

Production of OMVs is no longer a feature of Gram-negative bacteria only; Gram-positive bacteria can also release membrane vesicles [101]. The vaginal Lactobacilli strains *L. crispatus* BC3 and *L. gasseri* BC12 produce vesicles that are over 100 nm in diameter, which decrease the adhesion of HIV-1 (Human immunodeficiency virus-1) to target cells and protect against viral entry [102]. This OMV pathway of viral defense involves host cells, in addition to better-known functions

of vaginal Lactobacilli against other bacteria, through the production of lactic acid and hydrogen peroxide.

For alcoholic liver disease, patients with cytolysin in the fecal microbiome, e.g., cytolysin expressed by some *Enterococcus faecalis* strains, have been reported to show higher mortality than patients without fecal cytolysin [103]. Bacteriophages that target cytolysin-expressing *Enterococcus faecalis* were shown in mice to reduce ethanol-induced liver injury and steatosis [103].

6.4 Summary

As a data-driven field of research, metagenomic studies discover many associations, which are Level 1 evidence on the way to causality. Randomized controlled trials (RCTs) and Mendelian randomization (MR) in large cohorts are providing Level 2 evidence for some of the associations (Table 6.1). Prospective cohorts and more basic research on the microbiome will add to our confidence in Level 3 evidence—counterfactuals, which will guide public health decisions. If our understanding of members of the human microbiome and their products are as thorough as some of the traditional pathogens, there would be little doubt in a causal role, even if the molecular mechanisms and the interactions are more complicated. However, things always need to be prioritized.

References

[1] Tortora GJ, Funke BR, CL CT, editors. Chapter 14 Principles of disease and epidemiology copyright. In: Microbiol. An introd. 10th ed. Pearson; 2010.

[2] Tu A-HT. 15.2 How pathogens cause disease—microbiology | OpenStax. In: Parker N, Schneegurt M, Lister PM, Forster B, editors. Microbiology. The American Society for Microbiology Press; 2016.

[3] Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. Annu Rev Physiol 2016;78:481–504. https://doi.org/10.1146/annurev-physiol-021115-105238.

[4] Levine R, Evers C. The slow death of spontaneous generation (1668–1859). Biotech Chronicles. https://webprojects.oit.ncsu.edu/project/bio183de/Black/cellintro/cellintro_reading/Spontaneous_Generation.html [Accessed 5 July 2021].

[5] Jaynes ET. Probability theory: the logic of science. Cambridge: Cambridge University Press; 2003.

[6] Pearl J, Mackenzie D. The book of why: the new science of cause and effect. 1st. New York: Basic Books; 2018.

[7] Ding T, Schloss PD. Dynamics and associations of microbial community types across the human body. Nature 2014;509:357–60. https://doi.org/10.1038/nature13178.

[8] Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 2019. https://doi.org/10.1038/s41564-018-0337-x.

[9] Ben-Ari Y. Is birth a critical period in the pathogenesis of autism spectrum disorders? Nat Rev Neurosci 2015;16:498–505. https://doi.org/10.1038/nrn3956.
[10] Apps PJ, Weldon PJ, Kramer M. Chemical signals in terrestrial vertebrates: search for design features. Nat Prod Rep 2015;32:1131–53. https://doi.org/10.1039/c5np00029g.
[11] Powell N, Walker MM, Talley NJ. The mucosal immune system: master regulator of bidirectional gut–brain communications. Nat Rev Gastroenterol Hepatol 2017;14:143–59. https://doi.org/10.1038/nrgastro.2016.191.
[12] Vujkovic-Cvijin I, Sklar J, Jiang L, Natarajan L, Knight R, Belkaid Y. Host variables confound gut microbiota studies of human disease. Nature 2020;2020:1–7. https://doi.org/10.1038/s41586-020-2881-9.
[13] Qi Q, Li J, Yu B, Moon J-Y, Chai JC, Merino J, et al. Host and gut microbial tryptophan metabolism and type 2 diabetes: an integrative analysis of host genetics, diet, gut microbiome and circulating metabolites in cohort studies. Gut 2021. https://doi.org/10.1136/gutjnl-2021-324053.
[14] Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature 2015;519:92–6. https://doi.org/10.1038/nature14232.
[15] Turk JN, Zahavi ER, Gorman AE, Murray K, Turk MA, Veale DJ. Exploring the effect of alcohol on disease activity and outcomes in rheumatoid arthritis through systematic review and meta-analysis. Sci Rep 2021;11:10474. https://doi.org/10.1038/s41598-021-89618-1.
[16] Kolber MA, Walls RM, Hinners ML, Singer DS. Evidence of increased class I MHC expression on human peripheral blood lymphocytes during acute ethanol intoxication. Alcohol Clin Exp Res 1988;12:820–3. https://doi.org/10.1111/j.1530-0277.1988.tb01353.x.
[17] Nicolas GR, Chang PV. Deciphering the chemical lexicon of host–gut microbiota interactions. Trends Pharmacol Sci 2019;40:430–45. https://doi.org/10.1016/j.tips.2019.04.006.
[18] Azizov V, Dietel K, Steffen F, Dürholz K, Meidenbauer J, Lucas S, et al. Ethanol consumption inhibits TFH cell responses and the development of autoimmune arthritis. Nat Commun 2020;11:1998. https://doi.org/10.1038/s41467-020-15855-z.
[19] Wu W, Sun M, Chen F, Cao AT, Liu H, Zhao Y, et al. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. Mucosal Immunol 2017;10:946–56. https://doi.org/10.1038/mi.2016.114.
[20] Cao Y, Lin W, Li H. Large covariance estimation for compositional data via composition-adjusted thresholding. J Am Stat Assoc 2018;1–45. https://doi.org/10.1080/01621459.2018.1442340.
[21] Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. PLoS Comput Biol 2012;8. https://doi.org/10.1371/journal.pcbi.1002687, e1002687.
[22] Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. Nat Rev Cardiol 2017;14:577–90. https://doi.org/10.1038/nrcardio.2017.78.
[23] Liu X, Tang S, Zhong H, Tong X, Jie Z, Ding Q, et al. A genome-wide association study for gut metagenome in Chinese adults illuminates complex diseases. Cell Discov 2021;7(1):9. https://doi.org/10.1038/s41421-020-00239-w.
[24] Jie Z, Xia H, Zhong S-L, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun 2017;8:845. https://doi.org/10.1038/s41467-017-00900-1.
[25] Liu X, Tong X, Zou Y, Lin X, Zhao H, Tian L, et al. Inter-determination of blood metabolite levels and gut microbiome supported by Mendelian randomization. BioRxiv 2020. https://doi.org/10.1101/2020.06.30.181438. 2020.06.30.

[26] Jiang X, Alfredsson L. Modifiable environmental exposure and risk of rheumatoid arthritis - current evidence from genetic studies. Arthritis Res Ther 2020;22. https://doi.org/10.1186/s13075-020-02253-5.
[27] Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013;498:99–103. https://doi.org/10.1038/nature12198.
[28] Zhong H, Ren H, Lu Y, Fang C, Hou G, Yang Z, et al. Distinct gut metagenomics and metaproteomics signatures in prediabetics and treatment-naïve type 2 diabetics. EBioMedicine 2019. https://doi.org/10.1016/j.ebiom.2019.08.048.
[29] Jie Z, Liang S, Ding Q, Li F, Sun X, Lin Y, et al. Dairy consumption and physical fitness tests associated with fecal microbiome in a Chinese cohort. Med Microecol 2021. https://doi.org/10.1016/j.medmic.2021.100038.
[30] Schüssler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, Zhou W, Mishra T, Mataraso S, et al. A longitudinal big data approach for precision health. Nat Med 2019;25:792–804. https://doi.org/10.1038/s41591-019-0414-6.
[31] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55–60. https://doi.org/10.1038/nature11450.
[32] Jackson M, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE, et al. Signatures of early frailty in the gut microbiota. Genome Med 2016;8:8. https://doi.org/10.1186/s13073-016-0262-7.
[33] Jie Z, Liang S, Ding Q, Li F, Tang S, Wang D, et al. A transomic cohort as a reference point for promoting a healthy gut microbiome. Med Microecol 2021. https://doi.org/10.1016/j.medmic.2021.100039.
[34] Png CW, Lindén SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, et al. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am J Gastroenterol 2010;105:2420–8. https://doi.org/10.1038/ajg.2010.281.
[35] Liu X, Tong X, Zhu J, Tian L, Jie Z, Zou Y, et al. Metagenome-genome-wide association studies reveal human genetic impact on the oral microbiome. bioRxiv 2021. https://doi.org/10.1101/2021.05.06.443017.
[36] Zhang Y, Gu Y, Ren H, Wang S, Zhong H, Zhao X, et al. Gut microbiome-related effects of berberine and probiotics on type 2 diabetes (the PREMOTE study). Nat Commun 2020;11:5015. https://doi.org/10.1038/s41467-020-18414-8.
[37] Cohen CR, Wierzbicki MR, French AL, Morris S, Newmann S, Reno H, et al. Randomized trial of lactin-V to prevent recurrence of bacterial vaginosis. N Engl J Med 2020;382:1906–15. https://doi.org/10.1056/NEJMoa1915254.
[38] Xu F, Fu Y, Sun TY, Jiang Z, Miao Z, Shuai M, et al. The interplay between host genetics and the gut microbiome reveals common and distinct microbiome features for complex human diseases. Microbiome 2020;8:145. https://doi.org/10.1186/s40168-020-00923-9.
[39] Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BAH, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 2016;535:376–81. https://doi.org/10.1038/nature18646.
[40] Mukherji A, Kobiita A, Ye T, Chambon P. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. Cell 2013;153:812–27. https://doi.org/10.1016/j.cell.2013.04.020.
[41] Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 2014;6. https://doi.org/10.1126/scitranslmed.3009759, 263ra158.
[42] Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. Science 2012;336:489–93. https://doi.org/10.1126/science.1219328.

[43] Xiao L, Feng Q, Liang S, Sonne SB, Xia Z, Qiu X, et al. A catalog of the mouse gut metagenome. Nat Biotechnol 2015;33:1103–8. https://doi.org/10.1038/nbt.3353.

[44] Rausch P, Basic M, Batra A, Bischoff SC, Blaut M, Clavel T, et al. Analysis of factors contributing to variation in the C57BL/6J fecal microbiota across German animal facilities. Int J Med Microbiol 2016. https://doi.org/10.1016/j.ijmm.2016.03.004.

[45] Wroblewski LE, Peek RM, Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev 2010;23:713–39. https://doi.org/10.1128/CMR.00011-10.

[46] Chen X-H, Wang A, Chu A-N, Gong Y-H, Yuan Y. Mucosa-associated microbiota in gastric cancer tissues compared with non-cancer tissues. Front Microbiol 2019;10:1261. https://doi.org/10.3389/fmicb.2019.01261.

[47] Bugaytsova JA, Björnham O, Chernov YA, Gideonsson P, Henriksson S, Mendez M, et al. *Helicobacter pylori* adapts to chronic infection and gastric disease via pH-responsive BabA-mediated adherence. Cell Host Microbe 2017;21:376–89. https://doi.org/10.1016/j.chom.2017.02.013.

[48] Poole AC, Goodrich JK, Youngblut ND, Luque GG, Ruaud A, Sutter JL, et al. Human salivary amylase gene copy number impacts oral and gut microbiomes. Cell Host Microbe 2019;25(4):553–564.e7. https://doi.org/10.1016/j.chom.2019.03.001.

[49] Zhu J, Tian L, Chen P, Han M, Song L, Tong X, et al. Over 50,000 metagenomically assembled draft genomes for the human oral microbiome reveal new taxa. Genomics Proteomics Bioinformatics 2021;18:2790. https://doi.org/10.1016/j.gpb.2021.05.001.

[50] Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, et al. Ectopic colonization of oral bacteria in the intestine drives T H 1 cell induction and inflammation. Science 2017;358:359–65. https://doi.org/10.1126/science.aan4526.

[51] Bajaj JS, Matin P, White MB, Fagan A, Golob Deeb J, Acharya C, et al. Periodontal therapy favorably modulates the oral-gut-hepatic axis in cirrhosis. Am J Physiol Liver Physiol 2018. https://doi.org/10.1152/ajpgi.00230.2018. ajpgi.00230.2018.

[52] Sobko T, Reinders CI, Jansson E, Norin E, Midtvedt T, Lundberg JO. Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite. Nitric Oxide 2005;13:272–8. https://doi.org/10.1016/j.niox.2005.08.002.

[53] Hata DJ, Smith DS. Blood group B degrading activity of *Ruminococcus gnavus* α-galactosidase. Artif Cells Blood Substit Immobil Biotechnol 2004;32:263–74. https://doi.org/10.1081/BIO-120037831.

[54] Tailford LE, Owen CD, Walshaw J, Crost EH, Hardy-Goddard J, Le Gall G, et al. Discovery of intramolecular trans-sialidases in human gut microbiota suggests novel mechanisms of mucosal adaptation. Nat Commun 2015;6:7624. https://doi.org/10.1038/ncomms8624.

[55] Henke MT, Kenny DJ, Cassilly CD, Vlamakis H, Xavier RJ, Clardy J. *Ruminococcus gnavus*, a member of the human gut microbiome associated with Crohn's disease, produces an inflammatory polysaccharide. Proc Natl Acad Sci U S A 2019;116:12672–7. https://doi.org/10.1073/pnas.1904099116.

[56] Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, et al. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. Nature 2013;502:96–9. https://doi.org/10.1038/nature12503.

[57] McGregor JA, French JI, Jones W, Milligan K, McKinney PJ, Patterson E, et al. Bacterial vaginosis is associated with prematurity and vaginal fluid mucinase and sialidase: results of a controlled trial of topical clindamycin cream. Am J Obstet Gynecol 1994;170:1048–59. discussion 1059-60 https://doi.org/10.1016/s0002-9378(94)70098-2.

[58] dos Santos Santiago GL, Tency I, Verstraelen H, Verhelst R, Trog M, Temmerman M, et al. Longitudinal qPCR study of the dynamics of *L. crispatus*, *L. iners*, *A. vaginae*, (sialidase positive) *G. vaginalis*, and *P. bivia* in the vagina. PLoS One 2012;7. https://doi.org/10.1371/journal.pone.0045281, e45281.

[59] Bacher P, Hohnstein T, Beerbaum E, Röcker M, Blango MG, Kaufmann S, et al. Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. Cell 2019. https://doi.org/10.1016/j.cell.2019.01.041.

[60] Boldock E, Surewaard BGJ, Shamarina D, Na M, Fei Y, Ali A, et al. Human skin commensals augment *Staphylococcus aureus* pathogenesis. Nat Microbiol 2018. https://doi.org/10.1038/s41564-018-0198-3.

[61] Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 2013;110:9066–71. https://doi.org/10.1073/pnas.1219451110.

[62] Seck EH, Senghor B, Merhej V, Bachar D, Cadoret F, Robert C, et al. Salt in stools is associated with obesity, gut halophilic microbiota and *Akkermansia muciniphila* depletion in humans. Int J Obes (Lond) 2019;43:862–71. https://doi.org/10.1038/s41366-018-0201-3.

[63] Liou AP, Paziuk M, Luevano J-M, Machineni S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 2013;5:178ra41. https://doi.org/10.1126/scitranslmed.3005687.

[64] Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 2017;23:850–8. https://doi.org/10.1038/nm.4345.

[65] Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med 2017;23:107–13. https://doi.org/10.1038/nm.4236.

[66] Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med 2019. https://doi.org/10.1038/s41591-019-0495-2.

[67] Chevalier C, Stojanović O, Colin DJ, Suarez-Zamorano N, Tarallo V, Veyrat-Durebex C, et al. Gut microbiota orchestrates energy homeostasis during cold. Cell 2015;163:1360–74. https://doi.org/10.1016/j.cell.2015.11.004.

[68] Yoon HS, Cho CH, Yun MS, Jang SJ, You HJ, Hyeong KJ, et al. *Akkermansia muciniphila* secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. Nat Microbiol 2021;1–11. https://doi.org/10.1038/s41564-021-00880-5.

[69] Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome–brain–β-cell axis to promote metabolic syndrome. Nature 2016;534:213–7. https://doi.org/10.1038/nature18309.

[70] Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U, et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet 2019;51:600–5. https://doi.org/10.1038/s41588-019-0350-x.

[71] De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metab 2016;24:151–7. https://doi.org/10.1016/j.cmet.2016.06.013.

[72] Wen C, Yan W, Mai C, Duan Z, Zheng J, Sun C, et al. Joint contributions of the gut microbiota and host genetics to feed efficiency in chickens. Microbiome 2021;9:126. https://doi.org/10.1186/s40168-021-01040-x.

[73] Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0070803, e70803.

[74] Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, et al. Gut microbiome development along the colorectal adenoma–carcinoma sequence. Nat Commun 2015;6:6528. https://doi.org/10.1038/ncomms7528.

[75] Liu P, Wu L, Peng G, Han Y, Tang R, Ge J, et al. Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. Brain Behav Immun 2019. https://doi.org/10.1016/j.bbi.2019.05.008.

[76] Zhu F, Ju Y, Wang W, Wang Q, Guo R, Ma Q, et al. Metagenome-wide association of gut microbiome features for schizophrenia. Nat Commun 2020;11:1612. https://doi.org/10.1038/s41467-020-15457-9.

[77] Xie H, Guo R, Zhong H, Feng Q, Lan Z, Qin B, et al. Shotgun metagenomics of 250 adult twins reveals genetic and environmental impacts on the gut microbiome. Cell Syst 2016;3:572–584.e3. https://doi.org/10.1016/j.cels.2016.10.004.

[78] Guo X, Li S, Zhang J, Wu F, Li X, Wu D, et al. Genome sequencing of 39 *Akkermansia muciniphila* isolates reveals its population structure, genomic and functional diverisity, and global distribution in mammalian gut microbiotas. BMC Genomics 2017;18:800. https://doi.org/10.1186/s12864-017-4195-3.

[79] Johansson MEV, Hansson GC. Immunological aspects of intestinal mucus and mucins. Nat Rev Immunol 2016;16:639–49. https://doi.org/10.1038/nri.2016.88.

[80] Ansaldo E, Slayden LC, Ching KL, Koch MA, Wolf NK, Plichta DR, et al. *Akkermansia muciniphila* induces intestinal adaptive immune responses during homeostasis. Science 2019;364:1179–84. https://doi.org/10.1126/science.aaw7479.

[81] Tett A, Huang KD, Asnicar F, Fehlner-Peach H, Pasolli E, Karcher N, et al. The *Prevotella copri* complex comprises four distinct clades underrepresented in westernized populations. Cell Host Microbe 2019. https://doi.org/10.1016/j.chom.2019.08.018.

[82] Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 2012;15:606–14. https://doi.org/10.1016/j.cmet.2012.01.024.

[83] Neinast MD, Jang C, Hui S, Murashige DS, Chu Q, Morscher RJ, et al. Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. Cell Metab 2019;29:417–429.e4. https://doi.org/10.1016/j.cmet.2018.10.013.

[84] Lim MT, Pan BJ, Toh DWK, Sutanto CN, Kim JE. Animal protein versus plant protein in supporting lean mass and muscle strength: a systematic review and meta-analysis of randomized controlled trials. Nutrients 2021;13. https://doi.org/10.3390/nu13020661.

[85] Duan Y, Guo Q, Wen C, Wang W, Li Y, Tan B, et al. Free amino acid profile and expression of genes implicated in protein metabolism in skeletal muscle of growing pigs fed low-protein diets supplemented with branched-chain amino acids. J Agric Food Chem 2016;64:9390–400. https://doi.org/10.1021/acs.jafc.6b03966.

[86] Yu D, Richardson NE, Green CL, Spicer AB, Murphy ME, Flores V, et al. The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. Cell Metab 2021;33:905–922.e6. https://doi.org/10.1016/j.cmet.2021.03.025.

[87] Ikeda K, Kinoshita M, Kayama H, Nagamori S, Kongpracha P, Umemoto E, et al. Slc3a2 mediates branched-chain amino-acid-dependent maintenance of regulatory T cells. Cell Rep 2017;21:1824–38. https://doi.org/10.1016/j.celrep.2017.10.082.

[88] He Q, Gao Y, Jie Z, Yu X, Laursen JMJM, Xiao L, et al. Two distinct metacommunities characterize the gut microbiota in Crohn's disease patients. Gigascience 2017;6:1–11. https://doi.org/10.1093/gigascience/gix050.

[89] de Groot P, Nikolic T, Pellegrini S, Sordi V, Imangaliyev S, Rampanelli E, et al. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. Gut 2020. https://doi.org/10.1136/gutjnl-2020-322630. gutjnl-2020-322630.

[90] Paun A, Yau C, Meshkibaf S, Daigneault MC, Marandi L, Mortin-Toth S, et al. Association of HLA-dependent islet autoimmunity with systemic antibody responses to intestinal commensal bacteria in children. Sci Immunol 2019;4. https://doi.org/10.1126/sciimmunol.aau8125, eaau8125.
[91] Vatanen T, Kostic AD, D'Hennezel E, Siljander H, Franzosa EA, Yassour M, et al. Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. Cell 2016;165:842–53. https://doi.org/10.1016/j.cell.2016.04.007.
[92] Hebbandi Nanjundappa R, Ronchi F, Wang J, Clemente-Casares X, Yamanouchi J, Sokke Umeshappa C, et al. A gut microbial mimic that hijacks diabetogenic autoreactivity to suppress colitis. Cell 2017;171:655–667.e17. https://doi.org/10.1016/j.cell.2017.09.022.
[93] Wegorzewska MM, Glowacki RWP, Hsieh SA, Donermeyer DL, Hickey CA, Horvath SC, et al. Diet modulates colonic T cell responses by regulating the expression of a *Bacteroides thetaiotaomicron* antigen. Sci Immunol 2019;4:eaau9079. https://doi.org/10.1126/sciimmunol.aau9079.
[94] Zhang X, Zhang D, Jia H, Feng Q, Wang D, Di Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015;21:895–905. https://doi.org/10.1038/nm.3914.
[95] Zhou C, Zhao H, Xiao X, Chen B, Guo R, Wang Q, et al. Metagenomic profiling of the pro-inflammatory gut microbiota in ankylosing spondylitis. J Autoimmun 2020;107:102360. https://doi.org/10.1016/j.jaut.2019.102360.
[96] Szymula A, Rosenthal J, Szczerba BM, Bagavant H, Fu SM, Deshmukh US. T cell epitope mimicry between Sjögren's syndrome antigen a (SSA)/Ro60 and oral, gut, skin and vaginal bacteria. Clin Immunol 2014;152:1–9. https://doi.org/10.1016/j.clim.2014.02.004.
[97] Greiling TM, Dehner C, Chen X, Hughes K, Iñiguez AJ, Boccitto M, et al. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. Sci Transl Med 2018;10. https://doi.org/10.1126/scitranslmed.aan2306, eaan2306.
[98] Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe 2018;23:716–24. https://doi.org/10.1016/j.chom.2018.05.003.
[99] Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017;357:1156–60. https://doi.org/10.1126/science.aah5043.
[100] Yang D, Chen X, Wang J, Lou Q, Lou Y, Li L, et al. Dysregulated lung commensal bacteria drive interleukin-17B production to promote pulmonary fibrosis through their outer membrane vesicles. Immunity 2019;50:692–706.e7. https://doi.org/10.1016/j.immuni.2019.02.001.
[101] Toyofuku M, Nomura N, Eberl L. Types and origins of bacterial membrane vesicles. Nat Rev Microbiol 2019;17:13–24. https://doi.org/10.1038/s41579-018-0112-2.
[102] Ñahui Palomino RA, Vanpouille C, Laghi L, Parolin C, Melikov K, Backlund P, et al. Extracellular vesicles from symbiotic vaginal lactobacilli inhibit HIV-1 infection of human tissues. Nat Commun 2019;10:5656. https://doi.org/10.1038/s41467-019-13468-9.
[103] Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature 2019. https://doi.org/10.1038/s41586-019-1742-x.