The human body is home to at least as many microbial cells as human cells [1]. However, the most salient characteristic of the interaction between microbes and the human body is not the number of cells involved, but their inextricable link with each other. The microbiome (i.e., the microbial communities living within our bodies) can affect metabolism, development, immunity, and other aspects of human health. Imbalances in the microbiome may alter an individual’s health status. The ‘hygiene hypothesis’ (see Glossary) suggests, for example, that loss of microbial diversity in the human microbiome may underlie the recent increase in the incidence of asthma [2].

Bacteria exert their effects not only locally where they reside, but also at other sites of the human body; they release molecules that travel to other tissues, such as the liver or brain, traditionally considered sterile (Box 1). It is estimated that gut microbial metabolites represent 10% of the metabolites found in mammalian blood [3] and their effects on hosts are only starting to be identified, as reviewed elsewhere [4]. As a result of these novel insights, animals are no longer considered autonomous units but, rather, ‘holobionts’, the ensemble of the host and its microorganisms [5,6].

Existing methods to modulate the microbiome can cause sweeping alterations (e.g., fecal microbiota transplantation, antimicrobial drugs, and prebiotics). These methods are inadequate for understanding how the microbial community is structured and which particular species, strains, or secreted proteins produce the observed effects. Existing methods are also not refined enough to fine-tune the microbiome quite broadly [8], as the FDA has also warned [9]. Antibiotics frequently used to treat infections cause broad-spectrum changes to microbiome communities and their overuse can lead to resistance [10], reducing the proportion of beneficial microbes within the community [11]. Prebiotics and dietary interventions that induce the growth of beneficial bacteria may be useful for some indications, but do not allow specific kinds of bacteria to be targeted [12]. However, current microbiome engineering is constrained by a lack of appropriate tools (Box 2). Thus, new strategies are being sought to determine the key mechanisms underlying microbiome-associated phenotypes and to pinpoint the physiological roles of specific bacteria. These strategies may allow long-lasting, effective therapies to be implemented for pathological conditions to which resident bacterial communities might contribute.

To broaden our understanding of microbiome-linked diseases and to create cell-based ‘factories’ for the in situ production of currently available or novel therapeutics [13], we need powerful and generalizable technologies to engineer commensal microbes. Here, we review the current state-of-the-art of microbiome engineering needed for understanding host–pathogen interactions and discuss some of the most pressing challenges in the field.
**The Gut Microbiome in Human Health**

Important questions in microbiome research include: how do commensal microbes causally contribute to disease states and how do we modulate these effects? To answer these questions, we need to know what makes a healthy microbiome. Is there such a thing as a universal healthy microbiome and, if so, can the communities within it be programmed on-demand to preserve a healthy status or to reverse disease?

The **core microbiome** is defined as the set of species that individuals have in common. Efforts such as the Human Microbiome Project [14] have identified factors that differentiate healthy from diseased microbiota and that could define a global human core microbiome (e.g., production of essential vitamins, glycosaminoglycan biodegradation, and ecological diversity) [15]. Strain profiles in core microbiomes remain stable over time and individuals have distinct strain profiles [16]. Moreover, such strains may be highly localized: site-specific subspecies clades can result from differentiation in a particular part of the body. For example, *Haemophilus parainfluenzae* differentiates into subspecies, giving rise to distinct clades in the supragingival plaque, buccal mucosa, and tongue dorsum [14]. In an analysis of fecal profiles from 4000 individuals, one study identified the main subspecies, giving rise to distinct clades in the supragingival plaque, buccal mucosa, and tongue dorsum [14]. Polysaccharide A (PSA) from the capsule of *Bacteroides fragilis* can induce MHCII-dependent CD4+ T cell expansion; PSA was the first identified nonpeptide antigen capable of exerting this kind of response and revealing a role for polysaccharides as antigens [130].

**Box 1. Physiological Roles of Gut Microbiota By-products**

The short chain fatty acid n-butyrate, the main product of gut bacterial fermentation of dietary fiber, can induce functional colonic Treg cells in mice via epigenetic upregulation of the Foxp3 gene [109]. n-Butyrate also acts as a modulator of colonic macrophages via histone deacetylase inhibition, which leads to downregulation of proinflammatory response in vitro and in vivo [110]. Trimethylamine (TMA) is produced by gut bacteria after they metabolize phosphatidylcholine from dietary components and can then be chemically modified in the liver to trimethylamine-N-oxide (TMAO). High plasma concentrations of TMAO have been associated with atherosclerosis and heart disease and have correlated with increased survival of patients suffering from chronic heart failure [128]. 4-Ethylphenylsulphate (4-EPS) is another metabolite derived from bacteria, thought to be a uremic toxin relevant to neurodevelopmental disorders, that can induce anxiety-like behavior in mice [129]. Polyamines, synthesized from arginine by the gut microbiota, are able to suppress IL-18 secretion in the mouse lumen, resulting in decreased production of epithelial antimicrobial peptides (AMPs) and, consequently, impacting the host microbiome profile and susceptibility to intestinal inflammation [114]. Polysaccharide A (PSA) from the capsule of *Bacteroides fragilis* can induce MHCII-dependent CD4+ T cell expansion; PSA was the first identified nonpeptide antigen capable of exerting this kind of response and revealing a role for polysaccharides as antigens [130].

**Box 2. Current Advances in the Genetic Domestication of Commensal Bacteria**

Most of the core human microbiome bacteria, Lachnospiraceae and Faecalibacterium, among many others [17], are currently intractable to genetic modification. The process of bringing these bacteria under control for useful applications is referred to as **genetic domestication**. We already have tools to domesticate *Bacteroides* [63], one of the most prominent bacterial genera in the human gut. Basic tools already being used in other organisms have been repurposed to operate in *Bacteroides* [e.g., synthetic promoters, ribosome-binding sites (RBS), inducible sensors, memory switches, and CRISPR interference (CRISPRi)] [63]. However, universal tools for engineering other commensal bacteria, including bacterial species that we cannot yet grow in the laboratory in predefined cultures, would be of great utility. Recently, the culturing of new strains has been facilitated by the use of cocultures, leading to the identification of quinones [131] and γ-aminobutyric acid [132] as growth factors for human gut bacteria.
Strain Acquisition and Stabilization of the Human Microbiome

One study determined that populations of bacteria become stable in children at the age of 3 years old, regardless of the variability of microbial composition in communities from very different backgrounds [20]. Early childhood events, such as the mode of birth [21], formula feeding [22], or severe acute malnutrition [23], have been deemed critical for strain acquisition, which can have important implications for adults. For example, Cesarean birth delivery has been associated with increased risk of allergic rhinitis and atopy relative to the risks associated with vaginal delivery [24].

Microbial strains, including ‘ancestral beneficial microbes’, may be eliminated from an individual’s microbiome because of antibiotic treatment or lifestyle changes [25], contributing to increasingly common modern disorders such as asthma and obesity. For instance, a reduction of dietary fiber in germ-free mice harboring a human microbiota led to loss of Bacteroidales and Clostridiales taxa over time, compared with a more traditional diet [26]. After colonizing mice with a human fecal community, the authors fed one group of mice with a Western diet (WD) and another group with a diet poor in simple carbohydrates and rich in fiber. An irreversible loss in microbial diversity in the microbiomes of mice fed with the WD was observed compared with a traditional one, lasting even 6 weeks after their diet was changed [26]. These observations were extended to human communities undergoing urbanization in Asia and adopting a WD, defined as a diet high in fat and simple carbohydrates, as well as poor in nutrients that sustain bacteria (such as the long-chain carbohydrates found in dietary fiber) [26,27].

It is currently challenging to restore diversity to an impoverished microbiome in human populations subjected to decades of use, over-use, and even misuse of antibiotics, and to diets detrimental to microbiome preservation and development. The main difficulty lies in the fact that we cannot precisely define the optimal composition of the microbiota for each individual. Even if these communities were predictable, there may be numerous ways to reconstitute them, such that they would likely vary between individuals depending on human genetics, along with the presence of, or exposure to, other bacteria. Perhaps techniques applied to restoration ecology [28] (e.g., limiting the spread of invasive species in favor of diversity) should be applied, considering the demonstrated success of these techniques in restoring diversity in complex environments.

The Influence of the Microbiota in Disease

Correlations between human microbiomes and disease have been studied with monocolonized or human microbiota-associated (HMA) mice (germ-free mice bearing a particular person’s microbiota in the gut) as model systems. Since the first use of HMA mice to examine the role of Firmicutes in obesity [29,30], other correlations have been found between microbiota and diseases. For example, the link between asthma and a reduction in the relative abundance of the gut bacterial genera Lachnospira, Veillonella, Faecalibacterium, and Rothia was demonstrated after reduction of airway inflammation in HMA mice inoculated with feces enriched in these four bacterial taxa [31]. In addition, human feces naturally enriched in Akkermansia from a multiple sclerosis (MS) discordant human twin, accelerated MS development in MS transgenic mice in comparison with mice inoculated with the corresponding feces from the healthy twin [32]. Another example constitutes the link between Parkinson’s disease (PD) and the increased abundance of Proteus spp., Bilophila spp., and Roseburia spp., together with a decrease in Lachnospiraceae, Rikenellaceae, and Peptostreptococcaceae [33]. Microbiota samples (harboring the composition and abundance of the mentioned taxa) from PD patients exacerbated physical impairments in a mouse model of PD (α-synuclein overexpression), compared with microbiota from healthy donors [33].

The vast majority of correlations established between microbiomes and disease originate at the level of bacterial genera, rather than species. There are exceptions, however: ulcers can be caused by Helicobacter pylori [34]; and colitis, by C. difficile [35]. Other exceptions include Akkermansia muciniphila and Acinetobacter calcoaceticus, which are both increased in MS patients compared with healthy individuals [36]. A. calcoaceticus is able to produce interferon (IFN)γ production via Th1 cell-mediated proinflammatory responses in germ-free monocolonized mice, whereas Parabacteroides distasonis
(reduced in MS patients in comparison with healthy donors) promotes an anti-inflammatory response involving CD4+CD25+ T cells and interleukin (IL)-10 FoxP3+ regulatory T cells (Tregs) in monocolonized mice relative to germ-free mice [36]. Recently, the species A. muciniphila is attracting the attention of researchers because it has been reported to ameliorate disease symptoms in a transgenic mouse model of amyotrophic lateral sclerosis [37] and because its supplementation in a progeria (advanced aging) mouse model increased lifespan relative to that of nonsupplemented mice [38].

These correlations between changes in the microbiota and human diseases suggest that biodiversity can underpin the stability and resilience of healthy microbial ecosystems. The redundancy of function present in diverse microbial populations suggests that even if a single species is lost, other species may reconstitute the missing biological function.

Current Tools and Approaches in Microbiome Engineering

The specific elimination of members of the microbiome by targeted antimicrobials remains a grand challenge in the field. Promising strategies to knock out specific bacteria include bacteriocins [39] and bacteriophages [40]. Despite their potential, only two bacteriocins are commercially available (nisin from Lactococcus lactis and carnocyclin A from Carnobacterium maltaromaticum). The use of engineered bacteriophages as antimicrobial agents to treat bacterial infections in humans [41,42] is presently limited to Russia, Georgia, and Poland [43].

Another strategy for engineering the microbiome is to use genetically domesticated commensal microbes as noninvasive tools to facilitate functional studies and to gain insights into what might be happening in situ [44]. Below, we discuss strategies for building new commensal engineering tools.

The Choice of Chassis: Engineering Host Microbes

Synthetic biologists use the term ‘chassis’ to refer to the type of cell that harbors and maintains the DNA constructs needed for a particular function. Decisions about which chassis to use for interactions with the microbiome rest on: (i) viability, (ii) colonization, (iii) localization, and (iv) genetic tractability [45]. Viability refers to whether the chassis will survive passage through the gastrointestinal tract. Colonization refers to whether the chassis will become part of the native gut microbiota. While colonization is required for long-term treatment of some chronic diseases, it may not be desirable for transient interventions. Examples of transient applications, all involving Escherichia coli, include the use of a thiosulfate-sensor to sense inflammation in the mouse gut [46] or the use of green fluorescent protein (GFP) to track E. coli location and persistence in the rat intestine [47]. Another example is the expression of two enzymes implicated in phenylalanine (Phe) internalization and degradation by E. coli to reduce toxic Phe accumulation in healthy Cynomolgus monkeys after an oral Phe dietary challenge [48] (Table 1). Localization refers to the specific region of the gut affected by the disease. For example, Bacteroides spp., which localize in the cecum and colon, might be used to treat ulcerative colitis, which only affects the large intestine; and Lactobacillus spp., found in the small intestine, might be used to treat Crohn’s disease, which can affect any part of the gastrointestinal tract [45,49]. Genetic tractability refers to whether the organism can feasibly be genetically modified by transformation, gene expression, activation, or other means [45].

Among the chassis currently being used, probiotic bacteria such as E. coli (the traditional workhorse of synthetic biology) and lactic acid bacteria (including Lactobacillus and Lactococcus) have been engineered to diagnose conditions in the gut and produce protein therapeutics in vivo (Tables 1 and 2). These probiotics sense and kill bacterial infections (Table 2), and the engineered immunomodulatory probiotics can produce anti-inflammatory cytokines, such as IL-10 [50–52], IL-2 [53], and transforming growth factor (TGF)-β [54], to counteract chronic gut inflammation (Table 2). Certain probiotics have been engineered to detect quorum-sensing molecules produced by pathogenic bacteria, for example, cholera autoinducer-1 [55], Staphylococcus autoinducer peptide-I [56], or Pseudomonas aeruginosa autoinducer N-acyl homoserine lactone [57]. The sensed molecule can activate the production of toxins, lysins, or antibiotic enzymes that kill the pathogen. A sophisticated ingestible micro-bio-electronic device (IMBED) that combines engineered probiotic sensor bacteria and ultra-low
power microelectronics has been developed to sense intestinal bleeding or markers of inflammation, such as thiosulfate, and to respond with detectable luminescence [58]. Some of these engineered probiotics have been or are being tested in clinical trials; one example is *L. lactis* transgenic bacteria expressing IL-10, a Phase I trial to test the clinical safety of LL-Thy12 treatment on ten Crohn’s disease patients with chronic inflammation [50]. Another example is the Phase I/IIa, ongoing, first-in-human, oral single and multiple dose-escalation, randomized, double-blinded, placebo-controlled study in healthy adult volunteers and adult subjects with phenylketonuria (70 participants) to evaluate the safety, tolerability, kinetics, and pharmacodynamics (primary outcome: incidence of treatment-emergent adverse events) of SYNB1618 *E. coli* Nissle (EcN) treatment [48] (NCT03516487).

<table>
<thead>
<tr>
<th>Disease targeted</th>
<th>Chassis</th>
<th>Model system</th>
<th>Compound sensed</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colitis</td>
<td><em>Escherichia coli</em></td>
<td>Microfluidic device</td>
<td>Peroxide</td>
<td>[134,135]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>Mouse intestinal explants</td>
<td>Nitric oxide</td>
<td>[136]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli Nissle 1917</em></td>
<td>Pigs</td>
<td>Heme</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli NGF-1</em></td>
<td>Mice</td>
<td>Tetraionate</td>
<td>[137]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli Nissle 1917</em></td>
<td>Mice</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli Nissle 1917</em></td>
<td>In vitro</td>
<td>Thiosulfate</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli NGF-1</em></td>
<td>Mice</td>
<td></td>
<td>[58,137]</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td><em>E. coli Nissle 1917</em></td>
<td>Mice</td>
<td>Acyl-homoserine lactone (3OC12HSL) Pseudomonas aeruginosa quorum-sensing signals</td>
<td>[57]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>In vitro</td>
<td></td>
<td>[138,139]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli Nissle 1917</em></td>
<td>In vitro</td>
<td></td>
<td>[140]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli K-12, MG1655</em></td>
<td>In vitro</td>
<td>Vibrio cholerae quorum sensing signals</td>
<td>[55,141]</td>
</tr>
<tr>
<td></td>
<td>Lactococcus lactis</td>
<td>Mice</td>
<td></td>
<td>[142]</td>
</tr>
<tr>
<td></td>
<td><em>E. coli BW25113</em></td>
<td>Mice</td>
<td>Fucose indicative of Citrobacter rodentium</td>
<td>[143]</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus reuteri</td>
<td>Mass Spectrometry</td>
<td>Autoinducer peptide-I of Staphylococcus aureus</td>
<td>[56]</td>
</tr>
<tr>
<td>Metastatic cancer</td>
<td><em>E. coli Nissle 1917</em></td>
<td>Mice</td>
<td>Liver metastasis</td>
<td>[144]</td>
</tr>
<tr>
<td>(including colorectal, breast, and pancreatic)</td>
<td>Streptococcus thermophilus</td>
<td>Mice</td>
<td>Lactose</td>
<td>[145]</td>
</tr>
<tr>
<td>Diet sensor</td>
<td><em>E. coli NGF-1</em></td>
<td>Mice</td>
<td>Anhydrotetracycline</td>
<td>[146]</td>
</tr>
<tr>
<td></td>
<td>Bacteroides thetaiotaomicron</td>
<td>Mice</td>
<td>Arabinogalactan</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice</td>
<td>IPTG*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice</td>
<td>Rhamnose</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Gut Bacteria Associated with Human Disease Diagnosis

*Abbreviation: IPTG, isopropyl-β-D-thiogalactopyranoside.*
Another interesting application is the use of engineered probiotic bacteria to prevent viral infections. For example, researchers have engineered a strain of *Lactobacillus* to use as a live biotherapeutic to prevent HIV-1 transmission [59]. This strain of *Lactobacillus* was designed to express neutralizing antibodies against epitopes found on the HIV-1 envelope, such as the gp120/CD4 complex. Conceptually, the anti-HIV-1 protein would inactivate the infectivity of the virus towards CD4+ T cells [59]. However, these probiotic bacteria cannot continuously deliver the desired therapeutic molecule at high doses because of their low abundance in the intestinal tract (e.g., *E. coli* or *L. lactis*) [60] and their inability to robustly colonize the gut [61] (e.g., EcN [48]). Therefore, the activity of these bacteria is often limited to transient effects: as the bacteria are eliminated from the body, their therapeutic effects may disappear [19].

To overcome these hurdles, traditionally intractable commensal bacteria have been proposed as an alternative chassis for putative microbiome therapeutics.

**Genetic Domestication of Intractable Commensal Microbes**

We detail below the latest advances in genetic tools for engineering intractable commensal organisms and discuss how this technology might be leveraged to acquire an increased understanding of basic mechanisms of immunity and the microbiome, as well as possible potential approaches for treating difficult diseases.

**General Strategies to Genetically Engineer Intractable Commensals**

Bacteria inhabiting the mammalian gut naturally transfer genes among themselves via bacteriophage (phage) or mobile genetic elements (MGEs) (i.e., plasmids, transposons) and integrated conjugative elements. Horizontal gene transfer (transduction, conjugation, and transformation) might be leveraged to introduce genetic constructs via MGEs into commensal microbes [62]. Genes encoding integrases, conjugative machinery, and transposases associated with MGEs can be identified and incorporated into domesticated mating partners (i.e., *E. coli*, *Bacillus subtilis*) [63] (Figure 1).

**Genetic Toolkits for Controlling Gene Expression in Commensal Microbes**

Tools for the programmable control of endogenous or artificial gene expression are available for model organisms such as *E. coli*; however, such tools do not yet exist for many commensals of interest. In addition, *E. coli*-derived standard genetic tools cannot always be used in other commensal bacteria because many of them are not compatible, or their performance is affected, when they are transferred to commensal organisms [45]. For example, in *Bacteroides*, sigma factor, the principal transcription initiation factor that enables the specific binding of RNA polymerase to promoters, binds to a unique consensus sequence found in position –33/–7 relative to the transcription initiation site, which differs from the analogous sequence in promoters of model organisms [45,64]. The ribosome-binding site (RBS) of *Bacteroides* is AT-rich [65] and its strength is more dependent on secondary structure than the RBS of *E. coli* [66]. The *Bacteroides* RBS is also predicted to be more dependent on interactions with ribosomal protein S1 of the 30S ribosomal subunit. Thus, the uniqueness of both the promoter and the RBS architecture excludes the possibility of transferring standard genetic tools from *E. coli* to *Bacteroides* [63].

To overcome this problem, specific tools have been created for *Bacteroides*. These bacteria belong to the phylum Bacteroidetes, one of the two dominant phyla in human stool [67] along with Firmicutes [68]. In contrast to other gut bacteria, *Bacteroides* spp. are an attractive chassis as they exhibit stable abundance and long-term colonization in humans [69]. As a proof-of-concept, researchers have used specific tools for engineering *Bacteroides* species to control the expression of reporter genes [63,70].

One type of tool is a gene transfer platform used to introduce genetic parts from one species into another. Promoters, RBSs, and transcription terminators can be introduced via gene transfer platforms (Figure 1) to create gene regulatory libraries that span multiple orders of magnitude in gene expression strengths, as undertaken for *Bifidobacterium* [71] and *Bacteroides* [63,70].
<table>
<thead>
<tr>
<th>Disease targeted</th>
<th>Chassis</th>
<th>Host phenotype</th>
<th>Results</th>
<th>Model systems</th>
<th>‘Therapeutics’ produced</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera</td>
<td><em>Escherichia coli</em> Nissle 1917</td>
<td>2–3-day-old suckling CD-1 mice</td>
<td>Increased survival from ingestion of <em>Vibrio cholerae</em>, reduced cholera toxin binding to the intestine, and <em>V. cholerae</em> infection relative to untreated controls.</td>
<td>Mice</td>
<td>CAI-1</td>
<td>[147]</td>
</tr>
<tr>
<td>Experimental autoimmune diseases</td>
<td><em>Lactococcus lactis</em></td>
<td>OVA TCR transgenic mice on a BALB/c background</td>
<td>Ovalbumin-specific tolerance.</td>
<td>Mice</td>
<td>Ovalbumin</td>
<td>[116]</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>NOD Abo DQ8 transgenic mice</td>
<td>Suppression of local and systemic DQ8-restricted T cell responses (DTH responses).</td>
<td>Mice</td>
<td>DQ8 gliadin epitope</td>
<td>[117]</td>
<td></td>
</tr>
<tr>
<td>Colitis</td>
<td><em>L. lactis</em></td>
<td>Crohn’s disease patients, IL-10–/– mice, DSS- and TNBS-induced colitis</td>
<td>Decreased weight loss, damage scores, and immune activation, relative to untreated controls.</td>
<td>Mice</td>
<td>IL-10</td>
<td>[50,51,115]</td>
</tr>
<tr>
<td></td>
<td><em>L. lactis</em></td>
<td>IL-10–/– mice, DSS-induced chronic colitis</td>
<td>Daily oral administration of anti-mTNF nanobodies secreting <em>L. lactis</em> resulted in local delivery at the colon and significantly reduced inflammation in mice relative to untreated controls.</td>
<td>Mice</td>
<td>Anti-TNFα nanobodies</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td><em>L. lactis</em></td>
<td>DSS-induced colitis</td>
<td>Decreased elastolytic activity and inflammation and restored intestinal homeostasis; protection from intestinal permeability and from the release of cytokines and chemokines.</td>
<td>Mice and <em>in vitro</em> human intestinal epithelial cells</td>
<td>Elafin</td>
<td>[148]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus casei</em></td>
<td>DSS-induced colitis</td>
<td>SOD produced by recombinant bacteria attenuated oxidative stress and inflammation in mice.</td>
<td>Mice</td>
<td>SOD</td>
<td>[149]</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus gasseri</em></td>
<td>IL-10–/– mice</td>
<td><em>L. gasseri</em> producing MnSOD significantly reduced inflammation (e.g., reduced infiltration of neutrophils and macrophages) relative to untreated mice.</td>
<td>Mice</td>
<td></td>
<td>[150]</td>
</tr>
<tr>
<td></td>
<td><em>Bifidobacterium longum</em></td>
<td>DSS-induced colitis</td>
<td>Engineered <em>Bifidobacterium</em> reduced inflammation relative to untreated mice based on inflammatory TNF-α, IL-1β, IL-6, and IL-8 and myeloperoxidase (MPO) concentrations, as well as histological damage in colonic tissues.</td>
<td>Mice</td>
<td></td>
<td>[151]</td>
</tr>
</tbody>
</table>

Table 2. Examples of Putative ‘Therapeutics’ Produced by Microbes in Experimental Models* (Continued on next page)
<table>
<thead>
<tr>
<th>Disease targeted</th>
<th>Chassis</th>
<th>Host phenotype</th>
<th>Results</th>
<th>Model systems</th>
<th>‘Therapeutics’ produced</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus plantarum/ L. lactis</strong></td>
<td>TNBS-induced colitis</td>
<td>Macroscopic and microscopic damage and MPO activity were reduced by SOD.</td>
<td>Rats</td>
<td></td>
<td>[152]</td>
<td></td>
</tr>
<tr>
<td><strong>Bacteroides ovatus</strong></td>
<td>–</td>
<td>–</td>
<td>In vitro</td>
<td>IL-2</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td><strong>Bacteroides ovatus</strong></td>
<td>DSS-induced colitis</td>
<td>Xylan-regulated recombinant bacteria had a significant therapeutic effect over weight loss, stool consistency, and rectal bleeding and reduced inflammation and neutrophil infiltration, expression of proinflammatory cytokines, and accelerated healing of epithelium and production of goblet cells.</td>
<td>Mice</td>
<td>KGF-2</td>
<td>[153]</td>
<td></td>
</tr>
<tr>
<td><strong>Bacteroides ovatus</strong></td>
<td>DSS-induced colitis</td>
<td>Xylan-regulated recombinant bacteria significantly improved colitis, showing superior efficacy compared with conventional steroid therapies. Clinical effects included: healing of colonic epithelium, reduction of cell infiltration and proinflammatory cytokines, and induction of production of mucin-rich goblet cells in colonic crypts.</td>
<td>Mice</td>
<td>TGF-β1</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td><strong>Type 1 diabetes</strong></td>
<td>L. lactis</td>
<td>NOD mice</td>
<td>Autoantigen-secreting recombinant bacteria used for tolerance induction in type 1 diabetes prevented protein degradation during gastrointestinal passage. In combination with short-course low-dose anti-CD3, this treatment stabilized insulitis, preserved functional β-cell mass, and restored normoglycemia in mice. It did not eliminate pathogenic effector T cells, but increased the presence of functional CD4⁺Foxp3⁺CD25⁺ regulatory T cells.</td>
<td>Mice</td>
<td>GAD-65 and IL-10</td>
<td>[52]</td>
</tr>
<tr>
<td><strong>L. lactis</strong></td>
<td>–</td>
<td>–</td>
<td>In vitro</td>
<td>Insulin</td>
<td>[154,155]</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Continued

(Continued on next page)
<table>
<thead>
<tr>
<th>Disease targeted</th>
<th>Chassis</th>
<th>Host phenotype</th>
<th>Results</th>
<th>Model systems</th>
<th>‘Therapeutics’ produced</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. lactis</td>
<td>NOD mice</td>
<td>Higher insulin concentrations and glucose tolerance.</td>
<td>Mice, rats</td>
<td>Glutamic acid decarboxylase, GLP-1</td>
<td>[52,156]</td>
<td></td>
</tr>
<tr>
<td>E. coli Nissle 1917</td>
<td>Mice fed with a high-fat diet/TallyHo mice, a polygenic mouse model of type 2 diabetes/obesity</td>
<td>Lower food intake, adiposity, insulin resistance, and hepatosteatosis/reduced weight gain.</td>
<td>Mice</td>
<td>NAPEs</td>
<td>[157]</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa infections</td>
<td>E. coli Nissile 1917</td>
<td>Streptomycin-treated mice</td>
<td>The engineered probiotic exhibited prophylactic and therapeutic activity, including biofilm inhibition, against P. aeruginosa during gut infection.</td>
<td>Caenorhabditis elegans and mice</td>
<td>Bacteriocin</td>
<td>[57]</td>
</tr>
<tr>
<td>E. coli infections</td>
<td>L. casei</td>
<td>Developed mice model</td>
<td>Expression of human lactoferrin (hLF) in recombinant bacteria protected the host against bacterial infection. After treatment, E. coli colony numbers in duodenal fluid were significantly lower and histopathological analyses of the small intestine showed both decreased intestinal injury and increased villus length.</td>
<td>Mice</td>
<td>Lactoferrin</td>
<td>[158]</td>
</tr>
<tr>
<td>Influenza virus infections</td>
<td>Salmonella Typhimurium</td>
<td>Immunogenized mice</td>
<td>A DNA vaccine using self-destructing Salmonella encoding influenza WSN virus HA antigen, delivered to mice induced complete protection against a lethal influenza virus challenge.</td>
<td>Mice</td>
<td>Hemagglutinin antigen</td>
<td>[118]</td>
</tr>
<tr>
<td>Listeria monocytogenes infections</td>
<td>Lactobacillus paracasei</td>
<td>–</td>
<td>–</td>
<td>In vitro</td>
<td>Listeria adhesion protein</td>
<td>[159]</td>
</tr>
<tr>
<td>L. lactis</td>
<td>BALB/c mice infected with L. monocytogenes</td>
<td>Protection against L. monocytogenes and CD8+ T cell induction against listeriolsyn O antigen (LLO).</td>
<td>Mice</td>
<td>LLO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>L. lactis</td>
<td>Linoleic acid-supplemented diet</td>
<td>Recombinant lactobacilli expressing linoleic acid isomerase modulated the fatty acid composition of host fat.</td>
<td>Mice</td>
<td>Linoleic acid isomerase</td>
<td>[160]</td>
</tr>
<tr>
<td>Gastrointestinal dysbiosis</td>
<td>E. coli</td>
<td>–</td>
<td>–</td>
<td>Microfluidic device</td>
<td>AI-2</td>
<td>[161]</td>
</tr>
</tbody>
</table>

Table 2. Continued

(Continued on next page)
Bacteroides, gene circuits with a 10 000-fold activation range for specific genes were built by mutagenizing wild type (WT) RBSs and using reporter genes as readouts to create RBS libraries with various translation initiation strengths [63].

Finally, gene expression can be regulated in gut commensal bacteria (e.g., with chemically inducible systems) to acquire exogenous control over in situ gene expression. Apart from the synthetic inducible systems recently adapted from well-studied model bacteria to Bacteroides [72] and Lactobacillus plantarum [73], another option is adapting endogenous inducible systems from commensal bacteria, as accomplished for systems regulating carbohydrate metabolism [74], two-component signal-transduction mechanisms based on homology [63,75], and endogenous inducible promoters [76], undertaken for Bacteroides [63].

As an example of endogenous inducible systems in commensal bacteria, one of the systems that controls utilization of the carbohydrate rhamnose was adapted using the previously identified PBT3763 promoter activated by the transcriptional activator RhaR, which is triggered in response to rhamnose [77]. A second strategy to expand the repertoire of endogenous inducible systems involved using the putative two-component systems of Bacteroides; these systems had been previously identified by transcriptomic studies. Here, the inducible systems for chondroitin sulfate and arabinogalactan were used and the functionality of the systems was tested with luminescent reporters [63].

Genome-editing tools such as CRISPR-Cas9 can facilitate genetic manipulation in commensals [78]. CRISPR interference (CRISPRi), which allows sequence-specific regulation of gene expression at the transcriptional level, has mainly been used to achieve transient gene silencing, but its application could be extended to the dynamic, inducible, and targeted knockdown of endogenous genes in commensal microbes for functional studies [63].

For example, in Bacteroides thetaiotaomicron, CRISPRi was used to modulate the endogenous BT1854 gene, encoding resistance to the cationic antibiotic polymyxin B [79]. This was achieved by constitutively expressing a custom single guide RNA (sgRNA) that would target the resistance gene for polymyxin B, so that once triggered the IPTG-induced expression of dCas9, by the presence of the inducer, would sensitize the cells to the polymyxin B antibiotic [63].

Another application for using CRISPRi in B. thetaiotaomicron involved targeting the hybrid two-component sensor BT1754 responsible for regulating fructose utilization (which augments bacterial gut colonization) [75,80]. When induced, Bacteroides growth was reduced relative to the uninduced control, in media in which fructose was the only carbon source [63]. CRISPRi was applied to B. thetaiotaomicron in order to subsequently regulate the expression of endogenous genes to tune resistance and metabolic profiles [75,80].
Applications: Modulating Microbe–Microbe and Host–Microbe Interactions to Study Function

Assessment of the stability of colonization and the functional activity of the genetic constructs in vivo is needed to ensure that genetic tools function in the context of complex, localized microbiota [63]. Loss-of-function and individual genetic mutant screens can be performed to identify essential genes and to address unanswered questions about the microbiota: specifically, the characteristics, determinants, and ecology underlying initial colonization, resilience (the ability to recover from a diseased state), and immune activation. A more complete mechanistic understanding might reveal the role of particular genes and metabolic factors in niche partitioning, as well as information about the persistence and impact of various probiotic, commensal, and pathogenic species. Identifying the bacterial genes required for establishing symbionts in the gut under various conditions might thus increase our understanding of the microbiome and eventually be used to rebuild a healthier, more resilient microbiome [80].

For example, one study used engineering tools to knock down in Bacteroides, a putative indole-producing tryptophanase gene, BT1492, assessing its role in producing the uremic toxin indoxyl sulfate [81]. These specific bacteria are now being incorporated into strategies such as rational dietary changes or antibiotic regimes to modulate the concentrations of circulating uremic solute, aimed at treating chronic kidney disease [81].

Germ-free rodents have become a standard model for studying microbe–microbe interactions, as they lack all the complex signals emitted by a microbiota present in conventional mice [82]. These models allow researchers to colonize mice with predetermined and previously characterized microbial consortia. This strategy, together with genetic engineering approaches, can reveal potential causative associations between microbiota imbalance and disease. However, germ-free mouse models have a number of limitations, such as harboring an underdeveloped immune system [82], thus curtailing the potential translation of findings in these models directly into real-world settings.

Nevertheless, these models have been used for several preliminary studies. Gut microbiota treated with antibiotics are enriched in Bacteroidetes [83]. In one study, the effects of antibiotic treatment on the ratio of bacteria were studied; researchers used E. coli to tune the amounts of an interspecies quorum sensing signal, autoinducer-2 (AI-2), in the gut of mice [83]. The study was performed with germ-free mice colonized with bacteria from the two main phyla, Bacteroidetes and Firmicutes, by inducing the expression of AI-2. The affected ratio of Firmicutes/Bacteroidetes was restored, with increased numbers of Firmicutes associated with health, relative to controls [83].

Microbiome Influences on the Host Immune System

As a barrier to pathogen invasion, the host immune system maintains a remarkable equilibrium with the microbiota via its innate and adaptive arms [84]. Nonimmune facets of the host, such as the mucus barrier and antimicrobial peptides (AMPs) produced by intestinal epithelial cells, also influence the composition and function of resident microbial communities [85–87]. Conversely, microbial communities can affect the host immune system (Figure 2). As new or modified strains colonize the host (e.g., gut, skin), the immune system might attack these microbes; or microbes might elicit (via cytokine secretion or dendritic cell function [88]) a heightened immune response relative to basal conditions, which might lead to tissue damage or autoimmunity. As systemic infections have been reported following probiotic treatment [89,90], it is important to characterize immune system–microbiome interactions to select the safest microbial strain for probiotic treatment or probiotic engineering, in addition to achieving the beneficial effect for the patient. Here, we note the most remarkable and recent evidence indicating how commensal bacteria influence the host immune system and we also highlight which of these properties could influence microbiome engineering.

Recent work has reported how 53 bacterial species that cover the five dominant phyla residing in the human gut could modulate the immune system when transferred to monocolonized germ-free mice [91]. The phyla were: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria.
The study revealed that most microbes elicited diverse and redundant immunologic phenotypes. The same phylum or genus of bacteria did not result in the same patterns of transcription or immunomodulation, and more than one type of bacterium was able to elicit a similar immune phenotypic change. In addition, certain immune events were observed for the first time; for example, *Veillonella* (belonging to the Firmicutes phylum) increased the frequency of IL-10-producing CD4+ T cells relative to germ-free mice and decreased IL-22-producing innate lymphoid cells (ILC) in the colon; *L. rhamnosus* markedly reduced plasmacytoid dendritic cell (pDC) numbers relative to those...
in germ-free mice; and F. varium triggered robust and wide-spread immune alterations, ranging from the inhibition of AMPs in the small intestine, to the reduction of colonic CD4+ and CD8+ T cell responses relative to the other strains tested in monocolonized mice [91].

The proinflammatory and anti-inflammatory response ratio resulting from exposure to microbiome bacteria has been extensively studied. Commensal segmented filamentous bacteria (SFB), a...
Clostridium-related clade that comprises nearly obligate symbionts [92], stimulate innate and adaptive immune responses and protect the host from pathogens. SFB have the unique ability to promote Th17 cell differentiation and to induce the secretion of IgA in children [93]. Both responses are part of the effector arm of the immune system, which combats infection. SFB have also been shown to protect mice against murine norovirus infection in a mouse model of colitis [94]. Altogether, the evidence suggests that SFB could protect against pathogens via immune activation and competitive exclusion, a means of resisting the encroachment of other species. However, an exacerbated Th17 response elicited by SFB has been shown to induce lung autoantibodies and autoimmunity during the prearthritic phase in a mouse model of arthritis (K/BxN transgenic) [95] (Figure 2B). Therefore, selecting SFB as chassis or probiotic (a clear example of an intractable commensal) to treat microbiome dysbiosis might potentially improve the colonization ratio, but strongly induce Th17; both activities should be taken into consideration in order to avoid possible side effects related to autoimmunity.

When examining anti-inflammatory Treg responses, the principal inducers are the commensal Bacteroides fragilis [96], some Clostridia strains [97], and zwitterionic capsular polysaccharides (ZPS), produced by B. fragilis, Streptococcus pneumoniae, and Bacteroides cellosiolyticus, among others [98]. One study has demonstrated that the expression of a ZPS (polysaccharide A or PSA) by B. fragilis was sufficient to alleviate colitis in an experimental Rag–/– mouse model, in which colitis was induced by Helicobacter hepaticus Rag–/– compared with untreated mice, in an IL-10-dependent manner [99]. In another study, a human mixture of 17 Clostridia strains from human microbiota belonging to clusters IV, XIVa, and XVIII (which lack virulence factors), was administered to specific pathogen-free mice and attenuated disease symptoms in models of 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis and ovalbumin-induced allergic diarrhea, relative to controls [100]. Thus, Bacteroides and Clostridium might be explored as potential anti-inflammatory probiotics to combat gut inflammatory diseases (Figure 2C).

Helminths have recently emerged as players of host–microbiome interactions. In mice, the nematode Nippostrongylus brasiliensis has been shown to significantly increase the abundance of Lactobacillaceae and decrease the abundance of Peptostreptococcaceae, Clostridiaceae, and Turicibacteraceae in the ileum, relative to uninfected animals [101]. In addition, N. brasiliensis can decrease the relative abundance of SFB in the intestine relative to uninfected mice via a Th2 IL-13-dependent inflammatory immune response; indeed, IL-13−/− infected mice fail to reduce SFB relative to WT infected mice [101]. Infection by the helminth Trichuris muris protects mice from intestinal abnormalities in a model of Crohn’s disease by inhibiting colonization of an inflammatory Bacteroides species [102]. In humans, Clonorchis sinensis, a helminth inhabiting the bile duct and gall bladder, can alter the composition of the gut microbiota, decreasing populations of Bacteroides and Bifidobacterium, while increasing the populations of Dorea and Variovorax relative to subjects lacking C. sinensis [103]. Helminth infections [104] might also weaken the immune response to vaccination, as observed for the Bacillus Calmette–Guérin (BCG) vaccine against tuberculosis. Thus, as helminths in the gut can inhibit bacterial colonization by inducing Th2 inflammatory mechanisms, it would be interesting to evaluate their role in niche competition (Figure 2C) because they could be implicated in the impaired colonization of some probiotic strains or species.

Other studies have investigated connections between the immune response to vaccination and the microbiota [105]. A systematic review reported that in half of the 26 articles analyzed, probiotics appeared to improve the humoral response to vaccines, with the strongest antibody induction for oral vaccination (against cholera, polio, rotavirus, and Salmonella Typhi) and parenteral influenza vaccination [106]. Another study showed that the presence of B. longum in stool samples of children was associated with improved humoral and T cell responses to BCG, polio, and tetanus toxoid vaccines, while an abundance of Enterobacteriales, Pseudomonadales, and Clostridiales in the stools was associated with lower vaccine responses in the children [107]. These results suggest that probiotics, in addition to their use in treating gut dysbiosis, might be manipulated and potentially used to improve vaccine efficacy.
Apart from microbial cells and their components, the microbiota can also influence the host immune system via microbial metabolites. The most well studied of such metabolites are short chain fatty acids (SCFAs), which comprise acetate, propionate, and butyrate and are produced from fermented fibers. SCFAs are well known for their anti-inflammatory properties, which they confer by inducing the production of prostaglandin E2, and have been implicated in gut epithelium maintenance in rats [108]. SCFA have also been found to elicit Treg activation in a mouse model of colitis (via adoptive transfer of CD4+CD45RBhi T cells in Rag1−/− mice) [109], and the in vitro downregulation of the secretion of proinflammatory mediators from colonic macrophages in mice [110]. As a result of these presumed beneficial properties, studies have reported that direct application of SCFAs to enemas can show clinical and histological improvement of active inflammatory bowel disease (IBD) and colitis [111] in humans. Commensal bacteria also release ATP, which, in massive concentrations, initiates acute and chronic inflammatory responses through Th17 activation in germ-free mice [112]. Polyamines from breast milk, synthesized by gut microbiota from arginine, can enhance postnatal maturation of intestinal CD8+, CD4+, natural killer (NK), and B cells from suckling rats [113]. Intestinal microbes also metabolize bile acids, released into the duodenum, giving rise to secondary products; one such secondary product is taurine, which can enhance the activation of the NLRP6 inflammasome and the production of IL-18 in the murine intestinal epithelium relative to untreated mice; this cascade, in turn, can support epithelial barrier maintenance [114].

### Box 3. Consortia Engineering for Microbiome Modulation

Microbial consortia are mutually dependent groups of bacteria (i.e., bacteria that live in symbiosis). Perhaps the most well-known use of consortia for therapeutic purposes is the use of fecal transplantation to treat *Clostridium difficile* infections [7]. However, microbial consortia are highly complex, massively interconnected, and often uncharacterized, which makes transplantation difficult to apply to other diseases. As complicated as it is to untangle the benefits that fecal transplantation may have in treating *C. difficile* infections, it is equally challenging to apply the procedure of fecal transplantation to other diseases because we lack an understanding of the mechanistic basis of microbial interactions and of the long-term effects of such interventions.

Currently, organisms interacting in a bacterial consortium cannot be genetically engineered *in situ* by conventional methods [133]. One approach for this may be to create synthetic consortia composed of a known cluster of bacteria. A combination of microbiome engineering tools (such as phages, antibiotics, probiotics, and diet) could be applied, depending on the target species. Additional studies aimed at revealing complex microbiome-host-microbe interactions taking place in microbial communities, as well as studies of niche colonization and underlying ecological principles, need to be undertaken to understand the basic principles of a healthy microbiome and to be able to reprogram these communities in a predictable fashion.

### Engineering the Microbiome to Modulate Host Immunity

A common strategy aiming to influence the immune system via microbiome engineering is to use probiotic bacteria to repair immunological pathologies, as in colitis. Several probiotics such as EcN and *L. lactis* have been programmed to express cytokines such as IL-10 [50,51,115], IL-2 [53], or TGF-β1 [54], to counteract exacerbated inflammation of TNBS-induced colitis in mice [50,51,115]. *L. lactis*, administered as a probiotic expressing autoantigens, can induce immune tolerance in autoimmune diseases, as described in mouse models (e.g., of diabetes) [52,116,117]. In addition, *Salmonella typhimurium* and *L. lactis* have been engineered to express antigens from pathogens such as influenza virus or *Listeria monocytogenes*, respectively [118,119] (Table 2). The intake of WT probiotics is another strategy to influence host immunity, but recent results have demonstrated that the efficacy of such an approach is highly variable depending on the microbiota makeup of each individual [19,91,120].

In addition, prebiotics such as nondigestible carbohydrates can stimulate the growth of gut bacteria (e.g., *Bifidobacterium*) with anti-inflammatory properties, and might be used to modulate microbiome properties. Specifically, the acetate produced by *Bifidobacterium* can be utilized by...
Figure 3. Current tools to treat dysbiosis include (upper left) antibiotic treatment resulting in the loss of beneficial commensals (e.g., *Bifidobacterium*) as well as harmful bacteria (e.g., *Clostridium difficile*), thus weakening the resilience of the host. Probiotic and prebiotic treatment increases the proportion of beneficial bacteria, which may bring the microbiome community back to a balanced state. Similarly, fecal microbiota transplants can restore the microbiome to a healthy state; however, such changes are not long-lasting. New tools are needed to efficiently treat dysbiosis (upper right panel). The development of specific antimicrobials such as bacteriophages or antimicrobial peptides (AMPs) might enable the elimination of harmful bacteria without disturbing other beneficial commensals. The use of colonized commensal probiotics might exert long-lasting effects, and the engineering of commensal probiotics might potentially eliminate harmful bacteria permanently. We already have tools (lower left panel) to simulate human mono- or multicolonization in germ-free mouse models. Such approaches may enable the elucidation of bacteria–disease correlations; however, knocking out...

(Key figure legend continued at the bottom of the next page.)
butyrate-producing bacteria; the increase in butyrate production can improve gut barrier integrity and gut immunity, as evidenced from in vivo amelioration of IBD or colitis (among other diseases) in experimental models, and reviewed in [121].

Another interventional approach is the use of bacteriophages or the consortia engineering (see Box 3 for more information about consortia engineering). A recent study found that virulent bacteriophages targeting adherent invasive E. coli could alleviate Crohn’s disease symptoms in mice [122].

Although there is strong evidence supporting the influence of the microbiome on immune-related diseases, we are far from being able to predict the outcome of clinical interventions. Indeed, we are still learning the underlying mechanisms of the reciprocal interactions between the immune system and the microbiome (i.e., in terms of composition and activity).

Challenges and Future Directions

Existing methods to unravel the mechanisms underlying microbe–microbe and host–microbiome interactions, such as 16S RNA or metagenomic sequencing and/or the use of monoclonized or HMA mice, provide a census of microbes or genes present in a community. This type of information can reveal correlations between microbial signatures and host disease; however, these methods generally do not reveal causation (Figure 3, Key Figure). The precise regulation of gene expression in commensal organisms could show, for example, how specific surface polysaccharides affect colonization [123], or how incorporating heterologous biochemical pathways can affect the maintenance of the gut microbiota [124] and its consequences for host health [63]. CRISPRi, a powerful tool to dynamically inactivate particular endogenous genes, could be used to find genes involved in bacterial maintenance [80] or interspecies interactions [125]. Gene regulatory modules could also be used to build ‘genetic circuits’ to alter or enhance the activity of particular microbes; for example, by allowing commensals to sense their environment, to store information in genomically encoded memory, and to report this information [63].

Engineered commensal bacteria may bear great potential as producers of in situ therapeutics [e.g., anti-tumor necrosis factor (TNF) antibodies [126]] to help address several unmet clinical needs. If the delivery of protein drugs to the intestinal mucosa is inefficient [127], such bacteria might be orally administered to produce drugs that resist degradation in situ, in the acid- and protease-rich upper gastrointestinal tract. Furthermore, systemic treatments, currently applied to treat IBD, autoimmune diseases, and cancer, frequently require high-dose injections of drugs that can lead to severe side effects and these obstacles might ideally be overcome using targeted enteric delivery via genetically engineered commensal bacteria, although we are a long way from achieving this. Aiming to find treatments for the rare inherited disease phenylketonuria in which Phe reaches toxic concentrations in the blood (causing intellectual disability), one study programmed E. coli Nissle 1917 to secrete enzymes that break down Phe when the bacteria reach the gut, under anoxic conditions [48]. In vivo studies showed that oral treatment with this engineered bacterium significantly inhibited the characteristic increases of Phe after a high protein intake in monkeys and mice (around 40%) [48], and the treatment is now in clinical trials (NCT03516487).

Ideally, multiple drugs delivered in situ with potential synergistic effects might be adjusted with microbiome engineering according to the patient’s need (Figure 3). This strategy might perhaps offer more efficient and cost-effective therapies than other current treatments, particularly for long-term conditions. Multifaceted approaches certainly represent a fruitful area of investigation (J. Ripka, Masters Thesis, Albert-Ludwigs-Universität, 2016).
Concluding Remarks

We describe new advances in microbiome engineering as a promising target to help treat complex human diseases. Current efforts are underway to precisely define the core human microbiome and to determine when and how the constituent strains are acquired and stabilized. Ultimately, the goal of this research is to define the elements that constitute a healthy microbiome and to identify basic parameters that need to be adjusted in dysbiotic states. The technologies described here have already enabled the elucidation of certain functional microbiome mechanisms, and we are confident that as the field develops, new tools will be invented to generate more noninvasive diagnostics, to understand the individual contributions of microbes to immunity, health, and disease (see Outstanding Questions). As these technologies evolve, we believe that research to engineer the microbiome may be most effective in gut microbiota formulations (including probiotic and commensal bacteria), aiming to tailor these to the individual. Indeed, technologies developed by synthetic biology may point the way to personalized medicine for microbiome-related diseases.

Acknowledgments

The Lu lab acknowledges financial support from: Defense Threat Reduction Agency (DTRA; E2045481 via George Mason University, HDTRA1-15-1-0050, and HDTRA1-14-1-0007), The Leona M. and Harry B. Helmsley Charitable Trust (3239), National Institutes of Health (NIH; 229825 via Massachusetts General Hospital and 4-R33-AI121669-04), Pfizer Incorporated (8500437439), U.S. Army Medical Research and Material Command (W81XWH-16-1-0565, W81XWH-17-1-0159, and W81XWH-18-1-0513), Space and Naval Warfare Systems Center (N66001-13-C-4025), Defense Advanced Research Projects Agency (DARPA; 152304.5106735.0006 and HR0011-15-C-0084), National Science Foundation (NSF; CCF-1521925 and DMR-1419807), Army Research Office (W911NF-17-2-0077), ARO-ISN UARC (W911NF-13-D-0001 T.O. 8), American Heart Association (229460), Human Frontier Science Program (LT000595/2017-L), Singapore-MIT Alliance for Research and Technology (S.M.A.R.T.), Breast Cancer Alliance, United States-Israel Binational Science Foundation (2017189), Amyotrophic Lateral Sclerosis (LT000595/2017-L), Singapore-MIT Alliance for Research and Technology (MISTI) - Spain La Caixa (2244102), and Pew Charitable Trusts (to M.E. Inda; 00030623). C.F.N. holds a Presidential Professorship at the University of Pennsylvania.

Disclaimer Statement


Resources

https://clinicaltrials.gov/ct2/show/NCT03516487

References

11. Rea, M.C., et al. (2011) Effect of broad- and narrow-spectrum antimicrobials on Clostridium difficile and...
Staphylococcus aureus derived AIP-I detection. ACS Synth. Biol. 7, 1229–1237
131. Fenn, K., et al. (2017) Quinones are growth factors for the human gut microbiota. Microbiome 5, 161
160. Rosberg-Cody, E., et al. (2011) Recombinant lactobacilli expressing linoleic acid isomerase can modulate the fatty acid composition of host adipose tissue in mice. Microbiology 157, 609–615