



The Promise of Personalized Medicine

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List of Abbreviations

AID	Anti-inflammatory diet
CGR	Cardiac glycoside reductase
EEN	Exclusive enteral nutrition
FMT	Fecal microbial transplantation
HMP	Human Microbiome Project
IBD	Inflammatory bowel disease
JIA	Juvenile idiopathic arthritis
RA	Rheumatoid arthritis
ReA	Reactive arthritis
SCFA	Short-chain fatty acids
SpA	Spondyloarthritis

Introduction

The promise of personalized medicine is that in the future, we will be able to take a biospecimen from a patient and use the information contained within to design the patient's therapy. One potential source of information is the human genome. Unfortunately, there are very few situations in which genetic testing can be used to guide therapy; rheumatologists are familiar with testing for thio-purine methyltransferase activity prior to starting

azathioprine, as patients with defective gene activity are at increased risk of drug toxicity [1].

It is intuitively apparent that the microbiota, which among the organisms therein have been estimated to contain 100 times as many genes as their human hosts [2], are highly likely to contain genes that can both promote a disease and effect response to therapy. An essential difference between microbial and human genetics is that only the former can easily be modified with today's technology. This ability to modify the microbiota offers both peril and promise to microbiota-based therapy. The peril reflects whether assessment of the microbiota at any particular point in time actually reflects any truth about the patient's microbiota or whether it merely reflects the microbiota on the particular day it is sampled, which could be unrecognizably different by the next day. The promise, of course, is that it can be changed. As rheumatologists, we are familiar with genes that increase the risk of a particular condition, such as the strong association between HLA-B27 and ankylosing spondylitis [3]. Alas, if a patient with AS is HLA-B27+, there is scant that can be done about this polymorphism beyond continuing to work to understand the mechanism underlying its association with the disease. In contrast, if the same patient carried an abundance of microbial genes that contributed to his risk of developing the disease, then there is cause for optimism that microbial gene therapy may provide a therapeutic option.

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Stability of Microbiota

A detailed discussion of the ontogeny of the microbiota is beyond the scope of this review and has been summarized elsewhere [4]. In brief, in children born vaginally, the initial microbiota is highly similar to that of the genitourinary tract of the mother [5]. It then undergoes successive maturational changes throughout early childhood until it reaches an adult state [4]. Early data from the Human Microbiome Project (HMP) indicated that the adult state is reached around 2–3 years of age [6], although a subsequent study indicated that even older children have distinct microbiota from adults [7]. Even so, it is highly likely that the pace of the changes of the microbiota slows down after age 2–3 years. However, formal assessments of the stability of the microbiota have not been performed in school-age children.

In contrast, several studies performed in adult subjects have queried the stability of the microbiota by evaluating samples taken from adults at two or more points in time. Costello et al. obtained baseline and repeat samples from multiple different habitats (skin, gut, oral cavity, hair, nostril, outer ear) at baseline and after 3 months [8]. With respect to habitats, the variation between habitats (e.g., comparing stool to mouth) was far greater than that within habitats (e.g., comparing the mouth of one subject versus that of another). This was also the situation with individual participants, comparing two samples from the same habitat in different participants versus two samples from the same habitat in the same subject. However, the passage of time did not greatly increase the distance between the samples.

Likewise, Faith et al. followed 37 adults over a period 296 weeks [9]. To quantify the similarity between the samples, they used the Jaccard index, which in this case measures the fraction of shared strains between an individual at baseline and any time in the future—in essence, it measures the extent of overlap in a Venn diagram. The Jaccard index started off at approximately 0.9 in the first post-baseline collection, indicating 90% similarity. This gradually fell over time, but even at the end of the 5+-year study period, the

Jaccard index was over 0.6, indicating 60% similarity. In contrast, two unrelated individuals had a Jaccard index of approximately 0.3.

The microbiota is so stable that it is a potential forensic tool. This was assessed by Curtis Huttenhower and colleagues at Harvard, who studied baseline and follow-up samples obtained from healthy adults through the Human Microbiome Project [10]. Their aim in this study was to assess whether an individual's baseline sample could be identified through sequencing of the second. For this study, they evaluated multiple habitats (feces, skin, etc.) as well as multiple informatics tools of querying the contents of the microbiota. It emerged that feces provided more stability than any of the other habitats. In addition, traditional sequencing approaches (e.g., 16S) did not yield as much accuracy as a marker-based approach, which takes into account the bacterial counterparts to genetic polymorphisms to compare baseline and follow-up samples. Using this marker approach from fecal samples, the authors demonstrated that the correct subject could be identified a respectable 80% of the time, with most errors consisting of failure to identify the correct subject (false negative) rather than incorrectly attributing one subject's sample to another (false positive).

Do the Microbiota Cause Illness?

In order for assessment of the microbiota to be useful as a clinical tool, one would likely argue it has to be both associated with and even causal of a disease state and/or predictive of response to therapy. In animal models of the disease, it is relatively easy to prove causality. For example, germ-free arthritis-prone animals do not develop HLA-B27 spondyloarthritis (SpA) [11], rheumatoid arthritis [12], multiple sclerosis [13], or gout [14]. Clearly, in these model systems, gut bacteria are necessary for the development of the disease. However, since a germ-free human system is obviously impossible, a direct pathogenic role for the microbiota cannot be evaluated in humans with the same degree of rigor that it can be studied in laboratory animals. Nevertheless,

the body of literature does provide some evidence of a pathogenic role, and that comes from the consistency of findings within a particular disease state. The discussion in section IV of this textbook provided numerous examples of associations of an altered microbiota with a specific disease. For example, several studies in patients with inflammatory bowel disease (IBD; Chap. 15) demonstrated decreases in one particular organism, *Faecalibacterium prausnitzii* [15, 16], which was also found to be present in decreased abundance in children with the related condition of SpA [17]. Another study demonstrated that low *F. prausnitzii* abundance predicted poor response to therapy in children with IBD [18]. In contrast, *Prevotella copri* was found to be overly abundant in two unrelated studies of newly diagnosed RA patients (Chap. 15) [19, 20] yet has not been identified in any other disease models. Abundance of *Bacteroides* species was linked to multiple categories of JIA (Chap. 17) [17, 21], as well as to type I diabetes [22, 23], yet is depleted in adult IBD [24] and adult RA [20]. That the same bacteria often emerge as being associated in the same direction in the same disease using different patient populations is highly suggestive of a pathogenic or protective role for these particular organisms in the specific diseases in question.

Furthermore, it can be argued that the microbiota need not be dysbiotic for therapeutic alterations in such to have a potential benefit. First, as will be discussed below, the microbiota can influence response to drug therapy, by enhancing either effectiveness or toxicity. Thus, an alteration in the microbiota may permit usage of a drug that might otherwise result in dose-limited adverse events. Also, alterations in the overall diversity of the microbiota—the breadth and depth of organisms present in a sample—have been linked to a variety of diseases, including irritable bowel syndrome [25], psoriatic arthritis [26], and IBD [27]. Altered diversity may even have a predictive capacity, such as in the case of patients undergoing bone marrow transplantation, where patients with low diversity were less likely to survive treatment, even after adjustments for multiple confounding factors [28]. Likewise, in children at risk

for developing type I diabetes, decreased bacterial diversity preceded the development of diabetes-associated antibodies [22]. Thus, therapeutic alterations in microbial diversity could provide benefit, even if no single organism can be identified as being deficient or overly abundant. Third, to the extent that bacteria differ in their metabolic capacities or effects on the immune system, there might be a family of organisms whose levels are normal in comparison with the general population, but for which it is the case that alterations in their levels can repair a metabolic or immunologic defect caused by some other source such as host genetics or an undefined environmental trigger. For example, certain bacteria are highly capable of producing short-chain fatty acids such as butyrate, which are generally considered to have anti-inflammatory effects (reviewed by [29]). Even if the levels of such bacteria are normal in comparison with the general population, enhancing the production of these metabolites might still be beneficial for the patient: their levels may be too low at an active disease site, or increased levels may be required to counteract an abnormal pro-inflammatory stimulus. Finally, alterations in the microbiota may be able to influence disease states through modulation of immune function. The microbiota has profound influences on multiple arms of the immune system. Some elements of the microbiota influence innate immunity through modulation of innate lymphoid cells as well as through binding to innate receptors such as the toll-like receptors and the nucleotide oligomerization domain among others [30]; *Bacteroides fragilis* can program regulatory T cells [31]; the mouse bacteria segmented filamentous bacteria generate Th17 T cells [32]; and helminths can promote Th2 function [33], a property which has enabled their therapeutic use in IBD [34, 35].

The Microbiota and Drug Metabolism

Many therapeutic compounds are either prodrugs that have to be metabolized to active moieties (e.g., sulfasalazine, mycophenolate mofetil, azathioprine) or are taken as active compounds that

are subsequently inactivated by enzymes within the liver (e.g., nonsteroidal anti-inflammatory drugs, cyclosporine, tacrolimus). As many of the microbial genes have metabolic functions, the human intestinal microbiota constitutes a reservoir of metabolic enzymes that puts our livers to shame. In-depth reviews about the role of the microbiota in drug metabolism are available [36, 37]. Briefly, the microbiota appears to influence the absorption or metabolism of a variety of medications, including several such as nonsteroidal anti-inflammatory drugs, methotrexate, and sulfasalazine, which are widely used in rheumatology [36]. The microbiota may influence the tolerability of one of the most widely used medicines in rheumatology, methotrexate. Folic acid supplementation has long been recognized to reduce AEs associated with this therapy [38]. The microbiome of young children is far more efficient than that of adults at producing endogenous folic acid [4]; one can only speculate as to whether this might in part be responsible for the observation that methotrexate tends to be better tolerated in children with JIA as compared to adults with RA [39]. Data presented at the 2016 American College of Rheumatology conference showed that 2 of 25 bacterial species tested were able to metabolize oral methotrexate into polyglutamated methotrexate [40], the active moiety of the drug.

However, it is a non-rheumatic medicine, digoxin, that may best illustrate how the microbiota might impact drug delivery. Digoxin has long been used to treat congestive heart failure and arrhythmias. Digoxin can be metabolized to the inactive form dihydrodigoxin through reduction of the lactone ring [41]. Pronounced interindividual variability in absorption of the active form has been recognized for over 40 years [42], and a role of the intestinal microbiota in the inactivation of digoxin was initially recognized in 1981 [43]. In 1983, it was hypothesized that a specific organism, *Eubacterium lentum* (now called *Eggerthella lenta*), may be responsible for interindividual variations in digoxin pharmacokinetics in vivo due to its in vitro ability to inactivate this medicine [44]. However, the authors of that study were not able to confirm that this organism was responsible for digoxin

inactivation in vivo, due to their observations that many subjects unable to reduce digoxin also had high *E. lentum* abundance. Using technology not available in 1983, Haiser et al. identified a genetic element that they termed the cardiac glycoside reductase (*cgr*) operon that was present only in *E. lenta* strains capable of inactivating digoxin [45]. This study also revealed that in vitro supplementation of the amino acid arginine resulted in decreased expression of the *cgr* operon and thus decreased digoxin inactivation. These in vitro observations also translated to decreased in vivo digoxin inactivation in mice fed a high-protein diet, as evidenced by higher active digoxin levels in high-protein- versus low-protein-fed mice. Thus, not only can a specific strain of a single organism predict response to a cardiac drug, but diet may also influence drug levels.

Therapeutic Alterations of the Microbiota

There are several potential ways to alter the microbiota: antibiotics, probiotics, diet, and fecal transplantation are the most widely discussed.

Antibiotics

Certain antibiotics have long been used in the treatment of IBD, particularly the postsurgical complication of pouchitis [46]. Otherwise, their role in the management of chronic inflammatory diseases appears to be limited. There are compelling reasons to limit use of antibiotics for autoimmune diseases, including induction of bacterial resistance, risks of development of *C. difficile* colitis, and availability of safer and more effective alternatives in the current era, but it is certainly worth exploring the data to see what can be learned from the previous era. Along those lines, the effectiveness of antibiotics in patients with SpA has been disappointing. The vast majority of the studies of antibiotics in SpA patients specifically included those with reactive arthritis (ReA), which by definition has a known or strongly suspected infectious trigger [47]. Despite this, a

meta-analysis published in 2013 showed that antibiotics as a whole were ineffective in the management of ReA [48]. Likewise, a single randomized trial of doxycycline in patients with other forms of SpA also yielded negative findings [49]. In contrast, as discussed in this textbook (Chap. 15), studies in adults with RA have often shown multiple classes of antibiotics to be of benefit, although the mechanism of benefit remains unknown.

Probiotics

There may be a place for probiotics in the management of ulcerative colitis, although as recently reviewed, the data are not compelling [50]. Overall, this line of therapy has shown the least promise as a therapeutic tool. For example, two studies conducted in adult SpA, one a randomized placebo-controlled study [51] and the other a study conducted over the Internet that used only patient-reported outcomes but was nevertheless a randomized controlled trial [52], both yielded negative findings. Likewise, a RCT performed in children with juvenile SpA demonstrated improvement in both arms, possibly attributable to therapeutic nonsteroidal anti-inflammatory drug usage, with no differences between the groups among a panel of clinical and immunologic outcomes [53]. The studies of probiotics in RA showed minimal improvement, discussed in (Chap. 15).

One very plausible reason for their failure to alter the disease course is that they do not necessarily succeed in altering the contents of the microbiota, as summarized by a meta-analysis [54]. Work performed in Gary Wu's lab in mice showed that pretreatment with polyethylene glycol (the standard washout used for colonoscopies) and antibiotics permitted uptake of an engineered microbiota, while mice exposed to the same organisms without any pretreatment did not demonstrate any changes in their microbiota [55]. Thus, future studies involving probiotics may need to deplete the existing microbiota prior to adding new organisms. However, even this step does not guarantee success. Even if the probiotics did alter the microbiota, it does not necessarily follow that the changes would be

beneficial. Prior to any large-scale probiotic study, proof-of-concept studies need to be performed to evaluate the effects of the intervention on the community structure (e.g., diversity), as well as abundances of specific organisms that may be relevant to the disease state. So far this has not been done.

Diet

There is an abundance of data indicating that dietary therapies can rapidly alter the microbiota [56, 57] as well as specific data on the effects of individual nutrients on the fecal microbiome or metabolome. Examples include increased production of fecal short-chain fatty acids following exposure to poorly digestible carbohydrates [58], decreased abundance of *Prevotella* following exposure to a high-fat diet [59], and increased *Bifidobacteria* with whey as compared with casein protein [60]. These measures have demonstrated benefit in animal models of inflammatory diseases [59, 61, 62], although there are mixed data in human conditions. As discussed elsewhere, studies of dietary therapy have not shown much promise. In IBD, the studies have been small and somewhat contradictory. There are some proponents of excluding complex carbohydrates from the diet of IBD patients on the grounds that the enzymes required for breaking down disaccharides may be impaired in patients with IBD [63] and thus the specific carbohydrate diet and the low fermentable oligo-, di-, and monosaccharide and polyol (commonly known as FODMAP) diet have gained some attention, although controlled studies with objective endpoints are lacking [64]. On the other hand, non-digestible carbohydrates are fermented in the colon to make short-chain fatty acids (SCFAs) [65], and organisms responsible for the production of SCFAs are generally depleted in patients with IBD [66], indicating a potential role for consumption of complex carbohydrates in patients with IBD. Indeed, high-fiber diets have also resulted in symptomatic improvement in patients with IBD, although, again, rigorous studies are lacking [67]. There has recently been success with an "anti-inflammatory diet (AID)"

in the management of IBD. Olendzki et al. introduced 40 adult patients with IBD to an AID enriched for lean meats, omega-3 fatty acids, fibers, fruits, and vegetables, also encouraging foods with soft textures [68]. In this retrospective study, the 11 subjects who completed the diet for 1 month reported decreased symptom severity scales for Crohn disease (CD) and ulcerative colitis, as appropriate, based upon their IBD diagnosis. Likewise, Sigall-Boneh et al. treated 34 pediatric and 13 adult patients with CD with a combination of enteral nutrition and their version of an AID for 12 weeks [69]. This diet excluded gluten, dairy, animal fat, processed meats, emulsifiers, canned goods, and packaged products containing an expiration date. They reported remission in 33 (70%) of the subjects, including 6 of 7 subjects who followed the AID without supplemental enteral nutrition.

One form of dietary intervention that has had some consistent success is exclusive enteral nutrition (EEN), which consists of administration of a liquid diet, typically a polymeric formula, which is typically administered via a nasogastric or gastrostomy tube due to poor taste [70]. Randomized controlled studies in children with IBD have shown EEN to be equally effective as compared to corticosteroids in the induction of remission [71], while it appears to be less effective in adults [64]. There is also a case series of EEN use in children with JIA, demonstrating effectiveness among the 7 (of 13) children who maintained the therapy for more than 2 weeks [72]. The same group has also reported changes in the fecal microbiome and metabolome in association with EEN use [73].

A recent study indicated that a subject's baseline microbiota may influence response to dietary interventions. Kang et al. obtained baseline and follow-up fecal specimens from 12 healthy adults administered controlled diets containing varying doses of capsaicin in order to assess its effects on a variety of metabolic functions [74]. Consistent with the work of Wu et al. [56], they found that the baseline microbiota could be clustered into one of two enterotypes: one driven by *Bacteroides* and the other by *Prevotella*. Overall, subjects with the *Bacteroides*

enterotype were far more sensitive to the metabolic effects of capsaicin as compared to those with the *Prevotella* enterotype. They also demonstrated more pronounced effects on the fecal microbiota and metabolome.

Another study that evaluated baseline patient factors and response to dietary intervention used baseline IgG4 antibodies against 16 nutrients, the rationale being that IgG4 antibodies reflect chronic antigenic exposure [75] and thus might reflect specific intolerance. A total of 98 subjects with CD were randomized to exclude either the nutrients against which they had the four highest IgG4 antibodies (intervention group) or the four lowest (control). The intervention group demonstrated statistically significant improvements in the short IBD quality of life score as well as in the Crohn's Disease Activity Index.

Finally, it bears mentioning that effects of diet on inflammatory diseases need not be limited to the microbiota. Certain foods may have a direct pro- or anti-inflammatory potential [76]. Additionally, proper nutrition may affect the nutritional status or weight of a subject, factors which themselves may have salutary effects on the disease state.

In summary, the following conclusions may be reached about dietary interventions on inflammatory diseases:

- Diet has the potential to alter the microbiota, which itself may alter the disease state.
- Diets that otherwise may be considered unhealthy may nevertheless have a beneficial effect on arthritis. An illustration is the ability of a high-fat diet to prevent development of a mouse model of auto-inflammatory bone disease [59]. Conversely, simply changing to a more healthful diet does not automatically translate to clinical benefits for a specific disease.
- As inflammatory diseases are chronic, potentially lifelong processes, dietary therapy will have to be acceptable to the patient and family for it to be sustained long term.
- Dietary therapy may need to be tailored to individual subjects based upon their disease state, baseline microbiota, and potentially other factors.

Fecal Microbial Transplant

Originally designed for treatment of recurrent *C. difficile* infection, fecal microbial transplant (FMT) is an effort to alter the fecal microbiota in a patient by replacing with one from a healthy individual. Feces are administered by gavage or rectally, although efforts are also in progress to introduce a defined consortium of bacteria that can act as a functional microbiota, thus avoiding the need for human donors and permitting use of capsules as the delivery vehicle. Although there are websites providing instructions on at-home FMT, comprehensive screening of potential donors is performed at medical centers. Following anecdotal reports of improvement in IBD [77, 78], randomized trials were conducted, showing mixed benefit [79, 80]. These studies have generally shown this procedure to be safe, although bacteremia caused by the introduced bacteria has been reported [81].

Targeting Individual Bacteria

All of the above approaches use broad strokes to alter the microbiota. Although this might be appropriate in some situations, such as in patients with recurrent *C. difficile* infection or in patients with a highly dysfunctional microbiota due to a combination of genetics, inflammation, and antibiotics, a directed approach may provide a safer and more effective means of providing microbial-based therapy. Some potential mechanisms of doing so were suggested in a recent review [82]. For example, Guo et al. tested a peptide with an antimicrobial moiety attached to a targeting moiety that was specific to the oral pathogen *Streptococcus mutans* [83]. A second potential approach would be use of bacteriophages targeting specific bacteria. As reviewed [84], this concept has been around for nearly one century, although it has yet to find widespread use in medicine. One limitation to both these approaches is that depleting one organism may have downstream effects on the abundances of multiple organisms, which either fill the niche of the depleted organism or were dependent upon the

depleted organism and subsequently decrease in abundance [83]. Finally, Kuntz and Gilbert also proposed designing therapeutics to target specific microbial enzymes, an approach that has the advantage that it would not result in community-wide changes to the microbiota [82]. To the extent that by-products of bacterial metabolism may be involved in the inflammatory process, as recently suggested by Stoll et al. with respect to the tryptophan pathway in juvenile SpA [85], blocking bacterial enzymes not otherwise present in humans may have the potential to ameliorate disease.

Peek into the Future

So what might microbiota-based personalized medicine look like? In some cases, the microbiota might assist with diagnosis. In others, at time of diagnosis, the microbiota could be sampled and subjected to amplicon (16S) sequencing or perhaps shotgun sequencing. Based upon this, a unique treatment plan might be designed, in which the goal would be to generate a microbiota that might be more healthful for that particular disease state. This might involve introduction of organisms that affect the metabolism of drugs used to treat the disorder; using dietary, probiotic, or even bacteriophage therapy to increase or decrease the abundance of specific organisms that are associated with the disease state; or simply increasing the fecal microbial diversity. Drug therapy targeting bacterial enzymes predisposing to an unfavorable metabolic milieu might also be contemplated. Situations with more severe dysbiosis might require more drastic measures, such as EEN or FMT.

One final consideration is cost. Monitoring the fecal microbiota may sound interesting, but is it feasible from an economic standpoint? Here at UAB, the cost of performing 16S sequence analysis inclusive of all steps from DNA preparation to bioinformatics analysis is approximately \$50/sample. This is equivalent to a complete blood count and metabolic panel, labs routinely ordered in medical practice. At approximately \$1000/sample, shotgun sequencing is substantially more

expensive, although it will likely fall in price in the future. Even at its current price, it is in line with numerous types of advanced testing, and to the extent that such testing could aid in the management of chronic diseases, it may well be worth its cost.

References

- Clunie GP, Lennard L. Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine. *Rheumatology (Oxford)*. 2004;43(1):13–8.
- Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59–65.
- Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. *Lancet*. 1973;1(7809):904–7.
- Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut Microbiome during the first year of life. *Cell Host Microbe*. 2015;17(6):852.
- Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010;107(26):11971–5.
- Yatsunencko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222–7.
- Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome. *Microbiome*. 2015;3:36.
- Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science*. 2009;326(5960):1694–7.
- Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, et al. The long-term stability of the human gut microbiota. *Science*. 2013;341(6141):1237439.
- Franzosa EA, Huang K, Meadow JF, Gevers D, Lemon KP, Bohannon BJ, et al. Identifying personal microbiomes using metagenomic codes. *Proc Natl Acad Sci U S A*. 2015;112(22):E2930–8.
- Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernandez-Sueiro JL, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med*. 1994;180(6):2359–64.
- Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*. 2010;32(6):815–27.
- Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature*. 2011;479(7374):538–41.
- Vieira AT, Macia L, Galvao I, Martins FS, Canesso MC, Amaral FA, et al. A role for gut Microbiota and the metabolite-sensing receptor GPR43 in a murine model of gout. *Arthritis Rheumatol*. 2015;67(6):1646–56.
- Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis*. 2009;15(8):1183–9.
- Machiels K, Joossens M, Sabino J, De Preter V, Arijis I, Eeckhaut V, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut*. 2013;63(8):1275–83.
- Stoll ML, Kumar R, Morrow CD, Lefkowitz EJ, Cui X, Genin A, et al. Altered microbiota associated with abnormal humoral immune responses to commensal organisms in enthesitis-related arthritis. *Arthritis Res Ther*. 2014;16(6):486.
- Shaw KA, Bertha M, Hofmekler T, Chopra P, Vatanen T, Srivatsa A, et al. Dysbiosis, inflammation, and response to treatment: a longitudinal study of pediatric subjects with newly diagnosed inflammatory bowel disease. *Genome Med*. 2016;8(1):75.
- Maeda Y, Kurakawa T, Umemoto E, Motooka D, Ito Y, Gotoh K, et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. *Arthritis Rheumatol*. 2016;68(11):2646–61.
- Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife*. 2013;2:e01202.
- Tejesvi MV, Arvonen M, Kangas SM, Keskitalo PL, Pirttila AM, Karttunen TJ, et al. Faecal microbiome in new-onset juvenile idiopathic arthritis. *Eur J Clin Microbiol Infect Dis*. 2015;35(3):363–70.
- Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, et al. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J*. 2011;5(1):82–91.
- Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med*. 2013;11:46.
- Zhou Y, Zhi F. Lower level of *Bacteroides* in the gut Microbiota is associated with inflammatory bowel disease: a meta-analysis. *Biomed Res Int*. 2016;2016:5828959.
- Durban A, Abellan JJ, Jimenez-Hernandez N, Salgado P, Ponce M, Ponce J, et al. Structural alterations of faecal and mucosa-associated bacterial communities in irritable bowel syndrome. *Environ Microbiol Rep*. 2012;4(2):242–7.

26. Scher JU, Ubeda C, Artacho A, Attur M, Isaac S, Reddy SM, et al. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. *Arthritis Rheumatol*. 2015;67(1):128–39.
27. Michail S, Durbin M, Turner D, Griffiths AM, Mack DR, Hyams J, et al. Alterations in the gut microbiome of children with severe ulcerative colitis. *Inflamm Bowel Dis*. 2012;18(10):1799–808.
28. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood*. 2014;124(7):1174–82.
29. Stoll ML. Gut microbes, immunity, and spondyloarthritis. *Clin Immunol*. 2015;159(2):134–42.
30. Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature*. 2016;535(7610):65–74.
31. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*. 2011;332(6032):974–7.
32. Farkas AM, Panea C, Goto Y, Nakato G, Galan-Diez M, Narushima S, et al. Induction of Th17 cells by segmented filamentous bacteria in the murine intestine. *J Immunol Methods*. 2015;421:104–11.
33. Hussaarts L, Yazdanbakhsh M, Guigas B. Priming dendritic cells for th2 polarization: lessons learned from helminths and implications for metabolic disorders. *Front Immunol*. 2014;5:499.
34. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. *Gut*. 2005;54(1):87–90.
35. Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology*. 2005;128(4):825–32.
36. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat Rev Microbiol*. 2016;14(5):273–87.
37. Jourova L, Anzenbacher P, Anzenbacherova E. Human gut microbiota plays a role in the metabolism of drugs. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2016;160(3):317–26.
38. Morgan SL, Baggott JE, Vaughn WH, Austin JS, Veitch TA, Lee JY, et al. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. *Ann Intern Med*. 1994;121(11):833–41.
39. Graham LD, Myones BL, Rivas-Chacon RF, Pachman LM. Morbidity associated with long-term methotrexate therapy in juvenile rheumatoid arthritis. *J Pediatr*. 1992;120(3):468–73.
40. Nayak RR, O'Loughlin C, Fischbach M, Turnbaugh PJ. Methotrexate is an antibacterial drug metabolized by human gut Bacteria [abstract]. *Arthritis Rheum*. 2016;68(suppl 10).
41. Robertson LW, Chandrasekaran A, Reuning RH, Hui J, Rawal BD. Reduction of digoxin to 20R-dihydrodigoxin by cultures of *Eubacterium lentum*. *Appl Environ Microbiol*. 1986;51(6):1300–3.
42. Greenblatt DJ, Smith TW, Koch-Weser J. Bioavailability of drugs: the digoxin dilemma. *Clin Pharmacokinet*. 1976;1(1):36–51.
43. Lindenbaum J, Rund DG, Butler VP Jr, Tse-Eng D, Saha JR. Inactivation of digoxin by the gut flora: reversal by antibiotic therapy. *N Engl J Med*. 1981;305(14):789–94.
44. Saha JR, Butler VP Jr, Neu HC, Lindenbaum J. Digoxin-inactivating bacteria: identification in human gut flora. *Science*. 1983;220(4594):325–7.
45. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium *Eggerthella lenta*. *Science*. 2013;341(6143):295–8.
46. Sokol H. Probiotics and antibiotics in IBD. *Dig Dis*. 2014;32(Suppl 1):10–7.
47. Carter JD. Reactive arthritis: defined etiologies, emerging pathophysiology, and unresolved treatment. *Infect Dis Clin N Am*. 2006;20(4):827–47.
48. Barber CE, Kim J, Inman RD, Esdaile JM, James MT. Antibiotics for treatment of reactive arthritis: a systematic review and metaanalysis. *J Rheumatol*. 2013;40(6):916–28.
49. Smieja M, MacPherson DW, Kean W, Schmuck ML, Goldsmith CH, Buchanan W, et al. Randomised, blinded, placebo controlled trial of doxycycline for chronic seronegative arthritis. *Ann Rheum Dis*. 2001;60(12):1088–94.
50. Derikx LA, Dieleman LA, Hoentjen F. Probiotics and prebiotics in ulcerative colitis. *Best Pract Res Clin Gastroenterol*. 2016;30(1):55–71.
51. Jenks K, Stebbings S, Burton J, Schultz M, Herbison P, Highton J. Probiotic therapy for the treatment of spondyloarthritis: a randomized controlled trial. *J Rheumatol*. 2010;37(10):2118–25.
52. Brophy S, Burrows CL, Brooks C, Gravenor MB, Siebert S, Allen SJ. Internet-based randomised controlled trials for the evaluation of complementary and alternative medicines: probiotics in spondyloarthropathy. *BMC Musculoskelet Disord*. 2008;9:4.
53. Shukla A, Gaur P, Aggarwal A. Effect of probiotics on clinical and immune parameters in enthesitis-related arthritis category of juvenile idiopathic arthritis. *Clin Exp Immunol*. 2016;185(3):301–8.
54. Kristensen NB, Bryrup T, Allin KH, Nielsen T, Hansen TH, Pedersen O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Med*. 2016;8(1):52.
55. Shen TC, Albenberg L, Bittinger K, Chehoud C, Chen YY, Judge CA, et al. Engineering the gut microbiota to treat hyperammonemia. *J Clin Invest*. 2015;125(7):2841–50.
56. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science*. 2011;334(6052):105–8.

57. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559–63.
58. Bindels LB, Neyrinck AM, Salazar N, Taminiau B, Druart C, Muccioli GG, et al. Non digestible oligosaccharides modulate the gut Microbiota to control the development of Leukemia and associated Cachexia in mice. *PLoS One*. 2015;10(6):e0131009.
59. Lukens JR, Gurung P, Vogel P, Johnson GR, Carter RA, McGoldrick DJ, et al. Dietary modulation of the microbiome affects autoinflammatory disease. *Nature*. 2014;516(7530):246–9.
60. McAllan L, Skuse P, Cotter PD, O'Connor P, Cryan JF, Ross RP, et al. Protein quality and the protein to carbohydrate ratio within a high fat diet influences energy balance and the gut microbiota in C57BL/6J mice. *PLoS One*. 2014;9(2):e88904.
61. Le Leu RK, Young GP, Hu Y, Winter J, Conlon MA, et al. *Dig Dis Sci*. 2013;58(12):3475–82.
62. Sprong RC, Schonewille AJ, van der Meer R. Dietary cheese whey protein protects rats against mild dextran sulfate sodium-induced colitis: role of mucin and microbiota. *J Dairy Sci*. 2010;93(4):1364–71.
63. Kakodkar S, Farooqui AJ, Mikolaitis SL, Mutlu EA. The specific carbohydrate diet for inflammatory bowel disease: a case series. *J Acad Nutr Diet*. 2015;115(8):1226–32.
64. Rummelle FM. Role of diet in inflammatory bowel disease. *Ann Nutr Metab*. 2016;68(Suppl 1):33–41.
65. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, de Roos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451–5.
66. Cao Y, Shen J, Ran ZH. Association between *Faecalibacterium prausnitzii* reduction and inflammatory bowel disease: a meta-analysis and systematic review of the literature. *Gastroenterol Res Pract*. 2014;2014:872725.
67. Brotherton CS, Taylor AG, Bourguignon C, Anderson JG. A high-fiber diet may improve bowel function and health-related quality of life in patients with Crohn disease. *Gastroenterol Nurs*. 2014;37(3):206–16.
68. Olendzki BC, Silverstein TD, Pursuitte GM, Ma Y, Baldwin KR, Cave D. An anti-inflammatory diet as treatment for inflammatory bowel disease: a case series report. *Nutr J*. 2014;13:5.
69. Sigall-Boneh R, Pfeffer-Gik T, Segal I, Zangen T, Boaz M, Levine A. Partial enteral nutrition with a Crohn's disease exclusion diet is effective for induction of remission in children and young adults with Crohn's disease. *Inflamm Bowel Dis*. 2014;20(8):1353–60.
70. Whitten KE, Rogers P, Ooi CY, Day AS. International survey of enteral nutrition protocols used in children with Crohn's disease. *J Dig Dis*. 2012;13(2):107–12.
71. Heuschkel RB, Menache CC, Megerian JT, Baird AE. Enteral nutrition and corticosteroids in the treatment of acute Crohn's disease in children. *J Pediatr Gastroenterol Nutr*. 2000;31(1):8–15.
72. Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in patients with juvenile idiopathic arthritis. *Clin Exp Rheumatol*. 2016;34(5):941–5.
73. Berntson L, Agback P, Dicksved J. Changes in fecal microbiota and metabolomics in a child with juvenile idiopathic arthritis (JIA) responding to two treatment periods with exclusive enteral nutrition (EEN). *Clin Rheumatol*. 2016;35(6):1501–6.
74. Kang C, Zhang Y, Zhu X, Liu K, Wang X, Chen M, et al. Healthy subjects differentially respond to dietary capsaicin correlating with the specific gut enterotypes. *J Clin Endocrinol Metab*. 2016;101(12):4681–9. [jc20162786](https://doi.org/10.1210/clinem.20162786).
75. Stapel SO, Asero R, Ballmer-Weber BK, Knol EF, Strobel S, Vieths S, et al. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI task force report. *Allergy*. 2008;63(7):793–6.
76. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*. 2014;17(8):1689–96.
77. Russell GH, Kaplan JL, Youngster I, Baril-Dore M, Schindelar L, Hohmann E, et al. Fecal transplant for recurrent *Clostridium difficile* infection in children with and without inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2014;58(5):588–92.
78. Zhang FM, Wang HG, Wang M, Cui BT, Fan ZN, Ji GZ. Fecal microbiota transplantation for severe enterocolonic fistulizing Crohn's disease. *World J Gastroenterol*. 2013;19(41):7213–6.
79. Suskind DL, Brittnacher MJ, Wahbeh G, Shaffer ML, Hayden HS, Qin X, et al. Fecal microbial transplant effect on clinical outcomes and fecal microbiome in active Crohn's disease. *Inflamm Bowel Dis*. 2015;21(3):556–63.
80. Suskind DL, Singh N, Nielson H, Wahbeh G. Fecal microbial transplant via nasogastric tube for active pediatric ulcerative colitis. *J Pediatr Gastroenterol Nutr*. 2015;60(1):27–9.
81. Quera R, Espinoza R, Estay C, Rivera D. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent *Clostridium difficile* infection. *J Crohns Colitis*. 2014;8(3):252–3.
82. Kuntz TM, Gilbert JA. Introducing the Microbiome into precision medicine. *Trends Pharmacol Sci*. 2016;38(1):81–91.
83. Guo L, McLean JS, Yang Y, Eckert R, Kaplan CW, Kyme P, et al. Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc Natl Acad Sci USA*. 2015;112(24):7569–74.
84. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, et al. Phage therapy in clinical practice: treatment of human infections. *Curr Pharm Biotechnol*. 2010;11(1):69–86.
85. Stoll ML, Kumar R, Lefkowitz EJ, Cron RQ, Morrow CD, Barnes S. Fecal metabolomics in pediatric spondyloarthritis implicate decreased metabolic diversity and altered tryptophan metabolism as pathogenic factors. *Genes Immun*. 2016;17(7):400–5.