



# The Microbiome: Past, Present, and Future

# 1

Matthew L. Stoll

## Abbreviations

AS	Ankylosing spondylitis
EEN	Exclusive enteral nutrition
IBD	Inflammatory bowel disease
JIA	Juvenile idiopathic arthritis
RA	Rheumatoid arthritis

---

## Past: Surprising Insights into Today's Microbial World

All disease starts in the gut.—Attributed to Hippocrates

In the 1670s, Antony van Leeuwenhoek was the first to describe the presence of bacteria, which he described as “animalcules of the most minute size which moved themselves about very energetically [1].” Very little progress was made toward identifying or characterizing bacteria over the next two centuries. Infectious agents had not, it appears, captured the attention of the scientific community until Louis Pasteur promoted the concept that germs can cause transmissible disease, and Pasteur

as well as Robert Koch further contributed to the field by developing techniques to culture bacteria [2]. As reviewed in 1911 [1], in the 1870s, two independent groups detected the presence of bacteria in stool. However, much of the work at the time, quite understandably, was focused on isolation of specific organisms associated with devastating diseases. Along those lines, there were some major discoveries at the time, including discovery of the bacteria causing anthrax in the blood of a dead animal accompanied by the demonstration that the disease could be transmitted through injection of the blood into a healthy animal as well as isolation and identification of the bacteria causing such diseases as tuberculosis, bacterial dysentery, and cholera [1]. Of note, the investigator who discovered both *Mycobacterium tuberculosis* in 1882 and *Vibrio cholerae* in 1884, Robert Koch, is still known today for his work proving pathogenicity of these bacteria.

Interest in the intestinal microbiota as a whole did not emerge until early in the twentieth century. Elie Metchnikoff had a rather dismal view of the microbiota, fearing that it released toxins into the systemic circulation that produced senility, and he therefore advocated altering the colonic microbiota [3]. An extreme method of doing so, which gained some attraction in the early twentieth century, was colectomy. There were some adherents to this belief, including Dr. Arbutnot Lane, who performed colectomy or colonic bypass for a variety of

---

M. L. Stoll  
Department of Pediatrics/Division of Rheumatology,  
University of Alabama at Birmingham,  
Birmingham, AL, USA  
e-mail: [mstoll@peds.uab.edu](mailto:mstoll@peds.uab.edu)

indications [4]. By the 1920s, this procedure had fallen out of favor [3].

A more nuanced view of the intestinal microbiota was offered by Arthur Kendall, who hypothesized that they were typically benign, unless the host is colonized with specific pathogenic agents [1]. That the intestinal microbiota was essential for the health of the host was initially demonstrated in 1915, through studies on germ-free chicks, which showed poor development of the germ-free animals starting at 10 days of life [5]. These observations resulted in the conclusion that “man has a bacterial population in his intestinal tract; that under normal conditions the organisms in the intestinal tract are fairly characteristic and constant; normally they are harmless; [and] *they may be protective* [5].”

In addition to work in germ-free animals, several further lines of current research into the microbiota had their start 100 years ago. One of them is the functional capacity of intestinal bacteria, which today is studied through such tools as shotgun sequencing of microbial DNA and mass spectroscopy of fecal and plasma metabolites. Ford initially noted that bacteria differ in their ability to metabolize carbohydrates and proteins, characterizing bacteria into two categories: fermenters (carbohydrates metabolizers) and putrifiers (protein metabolizers) [6]. Kendall extended these findings, observing that “Food largely determines the type of intestinal bacteria [1].” Specifically, diets rich in carbohydrates resulted in the generation of bacteria with increased capacity to metabolize carbohydrates. Today, it is well recognized that fiber-rich diets result in increased abundance of bacteria capable of metabolizing complex carbohydrates [7]. While carbohydrate and protein metabolism were the focus of attention in the first two decades of the twentieth century, by mid-century, the microbial effects on multiple other endogenous substances were studied, including B-complex vitamins [8–10], vitamin C [11], and cholesterol [12].

Another area of active interest today that had its roots 100 years ago is interest in treating disease through alterations in the intestinal microbiota. While today’s efforts, as will be seen throughout this textbook, focus on the treatment

of chronic inflammatory diseases, interest in the pre-antibiotic era was in the management of infectious diseases. As discussed above, colectomy was an extreme method of altering the intestinal microbiota, but not the only one. Diet has long been recognized as a very effective means of doing so, beginning with observations from 1911 that bottle-fed and breast-fed infants had substantially different microbial populations, with these studies even showing increased “homogeneity” of the intestinal microbiota in bottle-fed infants [1]. These observations are a precursor to recent findings showing decreased alpha diversity in bottle-fed compared to nursed infants [13]. Torrey as well noted that diet strongly influenced the contents of the microbiota, writing “It has been my experience that the intestinal flora of dogs reacts very promptly and with great uniformity to changes in diet [14].” Kendall proposed using simple sugars to alter the microbiota as a therapeutic tool for bacterial dysentery, thus in effect introducing the first instance of a therapeutic prebiotic [1]. Lane followed therapeutic colectomies in the first decades of the twentieth century with introduction of pure cultures of bacteria, first *Lactobacillus bulgaricus* and later *Lactobacillus acidophilus*, an early use of probiotics [3]. In perhaps the first published fecal microbial transplant, Dalton transplanted *Escherichia coli* from a healthy subject to a child undergoing antibiotic therapy for meningitis, reporting that rectal but not oral administration of the organism resulted in successful uptake and may have contributed to resolution of the illness [15]. In 1955, Winkelstein evaluated *Lactobacillus acidophilus* as a therapeutic agent in 53 subjects with a variety of intestinal disorders, including ulcerative colitis, reporting mixed results [16]. For the most part, however, interest in probiotics remained low until the 1990s [3].

Loss of interest in probiotic therapy as a tool to alter the microbiota may have been due to the development of antibiotics, with penicillin introduced in 1928 and many others to follow. Improved public health measures in developed nations, including vaccinations and improved hygiene, likely also dampened enthusiasm in research into microbial-based therapy of intestinal

infections. In any event, the widespread use of antibiotics spurred interest in the 1940s and 1950s on the effect of these therapies on the contents of the intestinal microbiota [17–20] and subsequently on the development of antibiotic resistance [21]. Another line of research in that era that pertained to antibiotics, which at the time was largely of interest to the agricultural field, were the effects of antibiotic therapy on the growth of livestock. Several studies demonstrated that young animals fed antibiotics demonstrated increased growth [22–24]. Observations that these growth-promoting effects of antibiotics did not occur in germ-free animals [25] and were associated with increased efficiency of absorption of dietary fatty acids [26] resulted in the conclusion that changes in the fecal microbiota mediated the increased weight gain of young animals treated with antibiotics [26]. Although this practice has fallen in disfavor due to concerns of transmission of antibiotic-resistant bacterial pathogens to humans, interest in the effects of antibiotics on growth remains, with a recent study showing that early exposure to antibiotics may be associated with an increased risk of childhood obesity [27].

One final theme that emerged in the 1950s and is germane to this textbook is the association of the intestinal microbiota with autoimmune diseases, including those not intrinsic to the gastrointestinal tract. Perhaps the first such study was published by Seneca, who reported increased total and coliform bacteria in the feces of 15 patients with UC as compared to four healthy controls [28]. Studies in the 1950s evaluated the intestinal microbiota in pediatric celiac disease [29] and acne [30]. Subsequent early studies on the intestinal microbiota were published in Crohn disease in 1969 [31], rheumatoid arthritis (RA) in 1966 [32], and ankylosing spondylitis (AS) in 1978 [33].

Ultimately, all of these efforts were limited by technology. For 100 years following the resurgence of interest in the intestinal microbiota, the only tool available to characterize them was culture, which we know today to be a highly inefficient means to characterize bacteria. It is often cited that only 20% of intestinal bacteria can be

cultured [34]. Although this number may be higher [35], many of these bacteria require specialized media, and anaerobic culture is also technically demanding. In 1977, Carl Woese introduced the concept of identifying bacteria according to their ribosomal 16S DNA sequence [36], and 10 years later he published an immense database of bacterial 16S sequences [37]. This permitted use of DNA probes to characterize bacterial communities, and this technology was used in studies of RA [38] and AS [39] to name but two. However, the real explosion in microbial DNA technology had yet to come.

---

### Present: “Democratization of Metagenomics”

The intestinal tract is a wonderfully perfect incubator and culture medium combined... It must be evident that the direction that this flora takes will not be without influence upon the host.—Arthur Kendall (1911)

The last 10 years has witnessed an explosion of research into the microbiota. A PubMed search of microbiome or microbiota identified nearly 40,000 publications, the vast majority of which are under 10–15 years old. This research has been enabled by advances not only in sequencing technology but primarily in computing power; indeed, a typical smartphone contains more than 100,000 times the computing power of those that launched the lunar mission in 1969. More recently, even the initial sequencing of the Human Genome Project costs over \$3 billion and took approximately 13 years, whereas today, the estimated cost of whole human exome sequencing is under \$1000 <http://www.genome.gov/sequencingcosts/> (accessed December 18, 2017). Due to the lower costs, investigators around the world are able to contribute to the field, a capacity that Jeff Gordon dubbed the “democratization of metagenomics [40].” These efforts around the world have been tremendously supported by massive centralized efforts to catalog the microbiota: the Human Microbiome Project in the United States [41] and Euro-HIT in Europe [42]. Thanks in no small part to these efforts, reference databases contain over

1.4 million bacteria and 53 thousand archaea [43] as of the end of 2016.

Much of the human work involving the microbiome consists of identifying differences in the microbiota between patient groups, e.g., those with versus without a particular disease. Such work is open to criticism that these differences are associative, but do not necessarily reflect a causal relationship. That is, the inflammatory milieu associated with a particular disease, or even its treatments, may result in alterations in the microbiota that are challenging to control for using comparison groups of healthy individuals. However, important work in animals and even in humans to some extent has shown the power of the microbiota to shape the disease, as well as the therapeutic potential of alterations of the microbiota.

Multiple animal models of inflammatory disease are attenuated or in some cases accelerated when the animals are raised in a germ-free setting, either in a true gnotobiotic facility or through treatment with broad-spectrum antibiotics. These include models of RA [44], ulcerative colitis [45], and chronic noninfectious osteomyelitis [46]. In each of these models, disease was highly attenuated in the germ-free state, and, furthermore, Koch's postulates of disease causation were partially established by recurrence of the disease when the microbiota were reintroduced into the animals.

A striking example of mediation of disease through the microbiota is the transfer of the obesity phenotype. Turnbaugh et al. studied mice that were genetically programmed to develop obesity based upon mutations in the gene coding for the satiety signal leptin [47]. Obese mice had increased *Firmicutes* in their intestines, findings typical in the obese state. Impressively, transfer of the fecal microbiota to germ-free mice resulted in increased weight gain among mice that received microbiota from obese as compared to lean mice. There were no differences in chow consumption, so this difference reflected increased energy harvest.

Another example is the HLA-B27 transgenic rat model of spondyloarthritis. Typically, transgenic rats develop a spontaneous arthritis, orchitis, and colitis.

When raised in a sterile environment, the rats are protected against arthritis and colitis [48]; however, disease recurs when the animals are exposed to a cocktail of bacteria that includes *Bacteroides vulgatus* [49].

Human studies as well demonstrate that the microbiota can impact inflammatory diseases. One interesting illustration of this came from research in infants at risk for type I diabetes mellitus based upon HLA types [50]. The investigators obtained serial fecal specimens from 33 at-risk children from birth through age 3 years, finding that changes in the contents of the fecal microbiota preceded development of clinical disease.

Similarly, a study of adults with newly diagnosed RA showed an expansion of a single organism, *Prevotella copri*, in 75% of newly diagnosed subjects, that was not seen in healthy controls or established patients [51]. The pathogenic nature of this species was further demonstrated by oral gavage of mice, which resulted in colitis.

Finally, the impact of the microbiota on human disease is illustrated by therapeutic responses to treatment, possibilities that are still in their infancy. While antibiotic [52] and probiotic [53] therapy have long been a mainstay of treatment of inflammatory bowel disease, there has been increasing interest in the potential role of fecal microbial transplantation [54]. Additionally, it is clear that dietary manipulation through the use of exclusive enteral nutrition (EEN) can induce remission of inflammatory bowel disease (IBD) as effectively as can corticosteroids [55, 56], and EEN has also been reported to be beneficial in children with juvenile idiopathic arthritis [57]. Although dietary changes can induce rapid shifts in the microbiome [58], it is not clear whether the beneficial effects of dietary changes are mediated through the microbiome or some other mechanism. It remains to be seen whether microbial manipulation will have similar effects in other diseases.

It is not at all surprising that alterations in the microbiota can impact inflammatory diseases. The microbiota is required for normal development of the immune system [59], and the intestinal microbiota in particular represents the largest mass of microbial antigen and adjuvant that is encountered in life, thus setting the stage

for marked effects on systemic and mucosal immune systems [60]. Indeed, antibodies directed against commensal microbial components are present and potentially pathogenic in a variety of autoimmune diseases, including IBD [61], spondyloarthritis [62], and RA [63].

Finally, it bears mentioning that certain microbiota may also be beneficial. Not only are certain bacteria generally considered protective (e.g., *Faecalibacterium prausnitzii* in IBD (Chap. 19)), but there is a body of literature that an entire class of organisms, helminth parasites, may also be protective against allergic or autoimmune diseases. The data in mice were summarized in a recent review [64]. Evidence that parasitic infection may be protective against allergy or autoimmunity is as follows: (a) A meta-analysis determined that current infection with an intestinal

parasite was associated with reduced risk of allergic sensitization [65]; (b) worldwide rates of multiple sclerosis and parasitic infestation show an inverse correlation [66]; and (c) in an area endemic for filarial parasites, patients with RA were significantly less likely to be infected as compared to healthy controls [67]; an observational study of multiple sclerosis patients demonstrated that helminth infection was associated with reduced disease progression [68]. It does bear mention, however, that some studies have shown contradictory data with respect to helminth infection and atopic diseases [69–71], and consequently not all investigators have been convinced by the epidemiologic data [72]. Additionally, interventional studies of live parasites in a variety of human autoimmune disorders have generally shown mixed results (Table 1.1).

**Table 1.1** Therapeutic trials of parasitic worms

Study	Patient population	Study design	Parasite	Outcome
<b>Allergic rhinitis</b>				
[79]	100 adults	RCT	<i>Trichuris suis</i>	No improvement in symptoms
[80]	100 adults	RCT	<i>Trichuris suis</i>	No changes in allergic reactivity
<b>Asthma</b>				
[81]	30 adults	RCT	<i>Necator americanus</i> larvae	No improvement in airway hyperreactivity
[82]	32 adults	RCT	<i>Necator americanus</i> larvae	No improvement in airway hyperreactivity
<b>Inflammatory bowel disease</b>				
[83]	4 adults with CD and 3 with UC	OL, uncontrolled	<i>Trichuris suis</i>	6/7 achieved remission for at least part of the study period
[84]	29 adults with CD	OL, uncontrolled	<i>Trichuris suis</i>	At week 24, 21/29 (72%) responded; 23/29 (79%) met criteria for remission
[85]	54 adults with UC	RCT	<i>Trichuris suis</i>	Favorable response seen in 13/30 (43%) in the treatment group versus 4/24 (15%) controls ( $p = 0.04$ ). Remission occurred in $\leq 10\%$ in both groups
[86]	36 adults with CD	RCT	<i>Trichuris suis</i>	Improvements in symptoms seen in placebo and treatment groups; no comparisons performed
<b>Multiple sclerosis</b>				
[87]	5 treatment-naïve adults	OL, uncontrolled	<i>Trichuris suis</i>	Decrease in number of new MRI lesions from 6.6 to 2; no change in self-reported symptoms
[88]	10 adults	OL, uncontrolled	<i>Trichuris suis</i>	Increase in number of new MRI lesions from 6 to 21
[89]	16 treatment-naïve adults	OL, uncontrolled	<i>Trichuris suis</i>	Nonsignificant improvement in MRI lesions; self-reported improvement in symptoms in 12/16

CD Crohn disease, MRI magnetic resonance imaging, OL open-label, RCT randomized controlled trial, UC ulcerative colitis

It is of particular interest that we have come full circle in our understanding that some of the chronic rheumatic diseases may have microbial causes. Over a century ago, C. Fred Bailey proposed that RA was likely caused by toxins elaborated by microorganisms, which potentially resided in the joints, nasopharynx, or gastrointestinal tract [73]. Sulfasalazine was developed as a therapeutic agent on the basis of this assumption that RA is an infectious disease [74]. Indeed, as discussed in the RA chapter (Chap. 15), there have been multiple successful trials of antibiotics in RA, yet by the late twentieth century, the notion that this was an infectious illness was abandoned, and the effectiveness of antibiotics was attributed to intrinsic anti-inflammatory effects of these agents [75]. Yet now, as shall be discussed as well in the RA chapter (Chap. 15), there is substantial evidence that specific microbes and their associated inflammatory properties underlie the disease.

---

### Future: Microbiota-Based Therapeutics or Prevention

A lack of knowledge of the normal intestinal bacteria and their relations will be a serious handicap in recognizing the abnormal bacteria and their relations... Arthur Kendall (1911)

Much work lies ahead to understand not only the contributory role of the microbiota to the disease but also the extent to which microbial manipulation may have therapeutic potential. As with any medication, this will require well-designed randomized studies to assess safety and efficacy. Many rheumatologists are familiar with the concept of a “window of opportunity” to treat an inflammatory disease. We are also familiar with the idea that the disease process begins long before the first symptom emerges, as illustrated by lupus-associated antibodies being formed years before the clinical onset of disease [76]. For diseases mediated by the microbiota, the window may be long before even the first disease manifestation. We will learn in the juvenile idiopathic arthritis (JIA) chapter (Chap. 17) of evidence that elevated fecal *Bacteroides* in JIA may reflect not intrinsic pathogenicity of this genus but altered

immune development on account of it. We are also learning that early childhood events affecting the gut microbiota may influence the risk not only of pediatric autoimmune disease but possibly even adult disease as well. Gordon proposed the concept of microbial prevention, such as administering probiotics to infants immediately after birth, or even to their mothers just before delivery [40]. Probiotic studies involving infants have shown benefit in reducing the risk of type I diabetes [77] and atopy [78]. Thus, the future of microbiota-based therapeutics may prove to be as much of a public health measure as therapeutic measures for individual diseases.

---

### References

1. Kendall AI. Certain fundamental principles relating to the activity of Bacteria in the intestinal tract. Their relation to therapeutics. *J Med Res.* 1911;25(1):117–87.
2. Smith KA. Louis pasteur, the father of immunology? *Front Immunol.* 2012;3:68.
3. Podolsky SH. Metchnikoff and the microbiome. *Lancet.* 2012;380(9856):1810–1.
4. Smith JL. Sir Arbutnot lane, chronic intestinal stasis, and autointoxication. *Ann Intern Med.* 1982;96(3):365–9.
5. Kendall AI. The Bacteria of the intestinal tract of man. *Science.* 1915;42(1076):209–12.
6. Ford WW. Classification of intestinal bacteria: (preliminary note). *J Med Res.* 1901;6(1):211–9.
7. Heinritz SN, Weiss E, Eklund M, Aumiller T, Louis S, Rings A, et al. Intestinal microbiota and microbial metabolites are changed in a pig model fed a high-fat/low-fiber or a low-fat/high-fiber diet. *PLoS One.* 2016;11(4):e0154329.
8. Jimenez Diaz C, Ales JM, Vivanco F. Symbiotic action of intestinal microbial flora; studies on nicotinic acid, pyridoxine, folic acid, and vitamin B12 synthesis by microbial flora in the enteric tract. *Bull Inst Med Res Univ Madr.* 1953;6(2–3):105–28.
9. Abdel-Salaam A, Leong PC. Synthesis of vitamin B(1) by intestinal bacteria of the rat. *Biochem J.* 1938;32(6):958–63.
10. Ellinger P, Abdel Kader MM. The nicotinamide-saving action of tryptophan and the biosynthesis of nicotinamide by the intestinal flora of the rat. *Biochem J.* 1949;44(3):285–94.
11. Esselen WB, Fuller JE. The oxidation of ascorbic acid as influenced by intestinal bacteria. *J Bacteriol.* 1939;37(5):501–21.
12. Wainfan E, Henkin G, Rittenberg SC, Marx W. Metabolism of cholesterol by intestinal bacteria in vitro. *J Biol Chem.* 1954;207(2):843–9.

13. Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. *BJOG*. 2015;123(6):983–93.
14. Torrey JC. The regulation of the intestinal flora of dogs through diet. *J Med Res*. 1919;39(3):415–47.
15. Dalton HW. Implantation of *B. coli* into the human intestine. *Ir J Med Sci*. 1951;308:384–6.
16. Winkelstein A. Lactobacillus acidophilus tablets in the therapy of various intestinal disorders: a preliminary report. *Am Pract Dig Treat*. 1955;6(7):1022–5.
17. Campos JV, Hoenen W, Costa A, Trabulsi L, Pontes JF. Changes in intestinal flora under tetracycline. *Gastroenterology*. 1958;34(4):625–35.
18. Anderson GW, Cunningham JD, Slinger SJ. Effect of terramycin and certain phenylarsonic acid derivatives on the growth and intestinal flora of Turkey poults. *J Nutr*. 1952;48(4):539–52.
19. Lipman MO, Coss JA Jr, Boots RH. Changes in the bacterial flora of the throat and intestinal tract during prolonged oral administration of penicillin. *Am J Med*. 1948;4(5):702–9.
20. Thomas AR, Levine M. Some effects of penicillin on intestinal bacteria. *J Bacteriol*. 1945;49(6):623–7.
21. Goldberg HS, Goodman RN, Lanning B. Low-level, long-term feeding of chlortetracycline and the emergence of antibiotic-resistant enteric bacteria. *Antibiot Annu*. 1958;6:930–4.
22. Stern JR, Mc GJ. Antibiotics and early growth of rats fed a soybean oil meal diet. *Arch Biochem*. 1950;28(3):364–70.
23. Berg LR, Bearse GE, Mc GJ, Miller VL. The effect of removing supplemental aureomycin from the ration on the subsequent growth of chicks. *Arch Biochem*. 1950;29(2):404–7.
24. Sieburth JM, Gutierrez J, Mc GJ, Stern JR, Schneider BH. Effect of antibiotics on intestinal microflora and on growth of turkeys and pigs. *Proc Soc Exp Biol Med*. 1951;76(1):15–8.
25. Forbes M, Park JT. Growth of germ-free and conventional chicks: effect of diet, dietary penicillin and bacterial environment. *J Nutr*. 1959;67(1):69–84.
26. Eyssen H, de Somer P. The mode of action of antibiotics in stimulating growth of chicks. *J Exp Med*. 1963;117(1):127–38.
27. Saari A, Virta LJ, Sankilampi U, Dunkel L, Saxen H. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. *Pediatrics*. 2015;135(4):617–26.
28. Seneca H, Henderson E. Normal intestinal bacteria in ulcerative colitis. *Gastroenterology*. 1950;15(1):34–9.
29. Anderson CM, Langford RF. Bacterial content of small intestine of children in health, in coeliac disease, and in fibrocystic disease of pancreas. *Br Med J*. 1958;1(5074):803–6.
30. Loveman DE, Noojin RO, Winkler CH Jr. Comparative studies of enteric bacterial flora in acne vulgaris. *J Invest Dermatol*. 1955;25(3):135–7.
31. Drasar BS, Shiner M. Studies on the intestinal flora. II. Bacterial flora of the small intestine in patients with gastrointestinal disorders. *Gut*. 1969;10(10):812–9.
32. Mansson I, Olhagen B. Intestinal Clostridium perfringens in rheumatoid arthritis and other connective tissue disorders. Studies of fecal flora, serum antitoxin levels and skin hypersensitivity. *Acta Rheumatol Scand*. 1966;12(3):167–74.
33. Ebringer RW, Cawdell DR, Cowling P, Ebringer A. Sequential studies in ankylosing spondylitis. Association of Klebsiella pneumoniae with active disease. *Ann Rheum Dis*. 1978;37(2):146–51.
34. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. *Science*. 2005;308(5728):1635–8.
35. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci U S A*. 2011;108(15):6252–7.
36. Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci U S A*. 1977;74(11):5088–90.
37. Woese CR. Bacterial evolution. *Microbiol Rev*. 1987;51(2):221–71.
38. Vahtovuo J, Munukka E, Korkeamaki M, Luukkainen R, Toivanen P. Fecal microbiota in early rheumatoid arthritis. *J Rheumatol*. 2008;35(8):1500–5.
39. Stebbings S, Munro K, Simon MA, Tannock G, Highton J, Harmsen H, et al. Comparison of the faecal microflora of patients with ankylosing spondylitis and controls using molecular methods of analysis. *Rheumatology (Oxford)*. 2002;41(12):1395–401.
40. Gordon JI. Honor thy gut symbionts redux. *Science*. 2012;336(6086):1251–3.
41. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207–14.
42. 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.
43. Schloss PD, Girard RA, Martin T, Edwards J, Thrash JC. Status of the archaeal and bacterial census: an update. *MBio*. 2016;7(3):e00201–16.
44. 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.
45. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell*. 2007;131(1):33–45.
46. Hubbard TD, Murray IA, Perdew GH. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. *Drug Metab Dispos*. 2015;43(10):1522–35.
47. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut

- microbiome with increased capacity for energy harvest. *Nature*. 2006;444(7122):1027–31.
48. 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.
  49. Dieleman LA, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, et al. *Lactobacillus GG* prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut*. 2003;52(3):370–6.
  50. Lahoti TS, John K, Hughes JM, Kusnadi A, Murray IA, Krishnegowda G, et al. Aryl hydrocarbon receptor antagonism mitigates cytokine-mediated inflammatory signalling in primary human fibroblast-like synoviocytes. *Ann Rheum Dis*. 2013;72(10):1708–16.
  51. 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.
  52. Wilson L, Arabshahi A, Simons B, Prasain JK, Barnes S. Improved high sensitivity analysis of polyphenols and their metabolites by nano-liquid chromatography-mass spectrometry. *Arch Biochem Biophys*. 2014;559:3–11.
  53. Li S, Pozhitkov A, Ryan RA, Manning CS, Brown-Peterson N, Brouwer M. Constructing a fish metabolic network model. *Genome Biol*. 2010;11(11):R115.
  54. Castagnini C, Luceri C, Toti S, Bigagli E, Caderni G, Femia AP, et al. Reduction of colonic inflammation in HLA-B27 transgenic rats by feeding Marie Menard apples, rich in polyphenols. *Br J Nutr*. 2009;102(11):1620–8.
  55. 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. *Inflam Bowel Dis*. 2014;20(8):1353–60.
  56. Soo J, Malik BA, Turner JM, Persad R, Wine E, Siminoski K, et al. Use of exclusive enteral nutrition is just as effective as corticosteroids in newly diagnosed pediatric Crohn's disease. *Dig Dis Sci*. 2013;58(12):3584–91.
  57. 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.
  58. 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.
  59. Abramowicz S, Susarla HK, Kim S, Kaban LB. Physical findings associated with active temporomandibular joint inflammation in children with juvenile idiopathic arthritis. *J Oral Maxillofac Surg*. 2013;71(10):1683–7.
  60. Saleh M, Elson CO. Experimental inflammatory bowel disease: insights into the host-microbiota dialog. *Immunity*. 2011;34(3):293–302.
  61. Targan SR, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology*. 2005;128(7):2020–8.
  62. Mundwiler ML, Mei L, Landers CJ, Reveille JD, Targan S, Weisman MH. Inflammatory bowel disease serologies in ankylosing spondylitis patients: a pilot study. *Arthritis Res Ther*. 2009;11(6):R177.
  63. Pianta A, Arvikar S, Strle K, Drouin EE, Wang Q, Costello CE, et al. Evidence for immune relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2016;69(5):964–75.
  64. Wu Z, Wang L, Tang Y, Sun X. Parasite-derived proteins for the treatment of allergies and autoimmune diseases. *Front Microbiol*. 2017;8:2164.
  65. Feary J, Britton J, Leonardi-Bee J. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. *Allergy*. 2011;66(4):569–78.
  66. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis. *Neurology*. 2006;67(11):2085–6.
  67. Panda AK, Ravindran B, Das BK. Rheumatoid arthritis patients are free of filarial infection in an area where filariasis is endemic: comment on the article by Pineda et al. *Arthritis Rheum*. 2013;65(5):1402–3.
  68. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. *Ann Neurol*. 2007;61(2):97–108.
  69. Cooper PJ, Chico ME, Platts-Mills TA, Rodrigues LC, Strachan DP, Barreto ML. Cohort profile: the Ecuador life (ECUAVIDA) study in Esmeraldas Province, Ecuador. *Int J Epidemiol*. 2015;44(5):1517–27.
  70. Lynch NR, Palenque M, Hagel I, DiPrisco MC. Clinical improvement of asthma after anthelmintic treatment in a tropical situation. *Am J Respir Crit Care Med*. 1997;156(1):50–4.
  71. Webb EL, Nampijja M, Kaweesa J, Kizindo R, Namutebi M, Nakazibwe E, et al. Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey. *Allergy*. 2016;71(8):1156–69.
  72. Briggs N, Weatherhead J, Sastry KJ, Hotez PJ. The hygiene hypothesis and its inconvenient truths about Helminth infections. *PLoS Negl Trop Dis*. 2016;10(9):e0004944.
  73. Bailey CF. The treatment of chronic rheumatic and rheumatoid arthritis by radiant heat and cataphoresis. *Br Med J*. 1909;1(2505):13–5.
  74. Mayberry J. The history of 5-ASA compounds and their use in ulcerative colitis – trailblazing discoveries in gastroenterology. *J Gastrointest Liver Dis*. 2013;22(4):375–7.
  75. O'Dell JR, Elliott JR, Mallek JA, Mikuls TR, Weaver CA, Glickstein S, et al. Treatment of early seropositive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. *Arthritis Rheum*. 2006;54(2):621–7.
  76. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development



- of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med.* 2003;349(16):1526–33.
77. Uusitalo U, Liu X, Yang J, Aronsson CA, Hummel S, Butterworth M, et al. Association of early exposure of probiotics and islet autoimmunity in the TEDDY study. *JAMA Pediatr.* 2016;170(1):20–8.
78. Zhang GQ, Hu HJ, Liu CY, Zhang Q, Shakya S, Li ZY. Probiotics for prevention of atopy and food hypersensitivity in early childhood: a PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore).* 2016;95(8):e2562.
79. Bager P, Arved J, Ronborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. *J Allergy Clin Immunol.* 2010;125(1):123–30 e1–3.
80. Bourke CD, Mutapi F, Nausch N, Photiou DM, Poulsen LK, Kristensen B, et al. *Trichuris suis* ova therapy for allergic rhinitis does not affect allergen-specific cytokine responses despite a parasite-specific cytokine response. *Clin Exp Allergy.* 2012;42(11):1582–95.
81. Feary J, Venn A, Brown A, Hooi D, Falcone FH, Mortimer K, et al. Safety of hookworm infection in individuals with measurable airway responsiveness: a randomized placebo-controlled feasibility study. *Clin Exp Allergy.* 2009;39(7):1060–8.
82. Feary JR, Venn AJ, Mortimer K, Brown AP, Hooi D, Falcone FH, et al. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. *Clin Exp Allergy.* 2010;40(2):299–306.
83. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am J Gastroenterol.* 2003;98(9):2034–41.
84. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. *Gut.* 2005;54(1):87–90.
85. 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.
86. Sandborn WJ, Elliott DE, Weinstock J, Summers RW, Landry-Wheeler A, Silver N, et al. Randomised clinical trial: the safety and tolerability of *Trichuris suis* ova in patients with Crohn's disease. *Aliment Pharmacol Ther.* 2013;38(3):255–63.
87. Fleming JO, Isaak A, Lee JE, Luzzio CC, Carrithers MD, Cook TD, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult Scler.* 2011;17(6):743–54.
88. Voldsgaard A, Bager P, Garde E, Akesson P, Leffers AM, Madsen CG, et al. *Trichuris suis* ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. *Mult Scler.* 2015;21(13):1723–9.
89. Fleming J, Hernandez G, Hartman L, Maksimovic J, Nace S, Lawler B, et al. Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: results of the HINT 2 clinical trial. *Mult Scler.* 2017. <https://doi.org/10.1177/1352458517736377>.